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25 September 1998

Darol E. Dodd, Ph.D.  
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RE: Update on Additional Items for 1613-002

Dear Dr. Dodd:

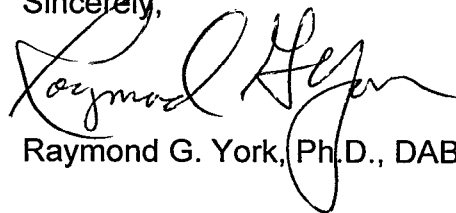
Below is an update on the status of the outstanding items for the above referenced study.

1. Missing individual data tables for APPENDIX M: This has been completed by Wright-Patterson Air Force Base..
2. Missing individual data tables for APPENDIX O: This has been completed by Wright-Patterson Air Force Base.
3. Additional morphometric analyses: Permission to examine the next lower dosage level has been received and the tissues will be sent to Dr. Garman early next week. The cost should not exceed \$2500 and the turn around time is 4-6 weeks.
4. Gender as a within-litter variable: There are two cases in which gender had an impact: one instance was found in the thyroid histopathology report generated by Wright-Patterson Air Force Base. And the other instance is within the motor activity data gathered by Argus Labs. This was for male pups on PN14 in 3 of 18 blocks - there was a significant increase in the number of movements, however, the overall number of movements was not significant. We will combine the male and female PN14 data and conduct a 3-way analysis. This will be

provided to you late next week.

5. Motor activity being re-analyzed as total counts per session: See response to question 4.
6. Table of rats sacrificed on PND 10 (faxed from USEPA): This table has been reviewed for accuracy. One animal number was found to be incorrect. The second occurrence of 19538 should be corrected to 19583. All other data are correct.
7. Historical control and validation data for passive avoidance, watermaze and auditory startle procedures are being provided at this time (Appendix 1-3). We are enclosing an article by Lochry et al. for the positive and negative control data for watermaze (Appendix 4).
8. Historical control data for motor activity will be provided late next week because we have three recently completed studies that need to be added to the historical control data. We are enclosing several studies and articles with positive and negative control data for motor activity (Appendix 5-10).
9. Historical control data for brain weight and morphometry measurements is being provided at this time (Appendix 11).
10. Data for brain weights for all dosage levels: This was already included in tables of final report.
11. Changes to graphs for body weight data: These graphs (with error bars) are in progress and will be provided late next week.

Sincerely,

A handwritten signature in black ink, appearing to read 'Raymond G. York', with a stylized flourish at the end.

Raymond G. York, Ph.D., DABT

APPENDIX 1  
PASSIVE AVOIDANCE HISTORICAL CONTROL

PASSIVE AVOIDANCE  
HISTORICAL CONTROL DATA  
Crl:CD@BR VAF/Plus@ RATS

CODE:	160.1	187	192
TYPE OF STUDY:	Seg. III	Seg. II/III	Seg. II/III
ROUTE:	Subcu.	Gavage	Gavage
FINAL DRAFT REPORT MAIL DATE:	11/24/95	07/23/96	08/30/96
TESTING DATES:	05/11/95 - 05/24/95	12/06/95 - 12/19/95	01/03/96-01/16/96

**PASSIVE AVOIDANCE LEARNING**

<u>MALE RATS: DAY 1</u>	N	24	23	23
TRIALS TO CRITERION	MEAN	5.2	5.0	4.3
	S.D.	1.6	2.4	0.9
LATENCY TRIAL 1	MEAN	5.3	7.6	6.6
	S.D.	3.0	4.9	4.0
LATENCY TRIAL 2	MEAN	33.8	20.7	33.6
	S.D.	21.4	17.6	22.5
FAILED TO LEARN	N	0	1	0
	%	0.0%	4.3%	0.0%

**PASSIVE AVOIDANCE RETENTION**

<u>MALE RATS: DAY 2</u>	N	24	22	23
TRIALS TO CRITERION	MEAN	2.8	2.9	2.8
	S.D.	0.9	0.6	0.5
LATENCY TRIAL 1	MEAN	35.0	27.4	26.4
	S.D.	23.8	23.0	22.7

**PASSIVE AVOIDANCE LEARNING**

<u>FEMALE RATS: DAY 1</u>	N	23	23	23
TRIALS TO CRITERION	MEAN	4.6	4.8	4.4
	S.D.	2.4	1.4	0.9
LATENCY TRIAL 1	MEAN	5.0	5.7	5.1
	S.D.	2.6	3.6	3.3
LATENCY TRIAL 2	MEAN	35.9	21.7	34.3
	S.D.	21.8	17.8	19.1
FAILED TO LEARN	N	1	0	0
	%	4.3%	0.0%	0.0%

**PASSIVE AVOIDANCE RETENTION**

<u>FEMALE RATS: DAY 2</u>	N	22	23	23
TRIALS TO CRITERION	MEAN	3.2	2.8	3.0
	S.D.	1.2	0.4	1.0
LATENCY TRIAL 1	MEAN	31.3	25.4	31.1
	S.D.	22.2	21.9	22.7



**PASSIVE AVOIDANCE  
HISTORICAL CONTROL DATA  
Crl:CD®BR VAF/Plus® RATS**

CODE:	228	234	242
TYPE OF STUDY:	Pre and postnat. dev.	Seg. II/III	Seg. II/III
ROUTE:	IV	Gavage	IV
FINAL DRAFT REPORT MAIL DATE:	01/23/97	03/05/97	04/09/97
TESTING DATES:	06/22/96 - 07/05/96	07/10/96 - 07/23/96	08/27/96 - 09/08/96

**PASSIVE AVOIDANCE LEARNING**

<u>MALE RATS: DAY 1</u>	N	24	24	24
TRIALS TO CRITERION	MEAN	4.4	4.6	5.4
	S.D.	1.0	1.0	2.5
LATENCY TRIAL 1	MEAN	11.7	10.4	9.0
	S.D.	5.3	9.1	6.6
LATENCY TRIAL 2	MEAN	35.0	30.0	21.3
	S.D.	22.8	21.4	18.8
FAILED TO LEARN	N	0	0	0
	%	0.0%	0.0%	0.0%

**PASSIVE AVOIDANCE RETENTION**

<u>MALE RATS: DAY 2</u>	N	24	24	24
TRIALS TO CRITERION	MEAN	2.1	2.8	3.1
	S.D.	0.3	0.6	1.0
LATENCY TRIAL 1	MEAN	56.9	30.7	24.3
	S.D.	10.6	24.4	22.2

**PASSIVE AVOIDANCE LEARNING**

<u>FEMALE RATS: DAY 1</u>	N	25	22	24
TRIALS TO CRITERION	MEAN	4.5	4.5	4.8
	S.D.	1.0	1.1	1.6
LATENCY TRIAL 1	MEAN	7.0	7.2	8.3
	S.D.	3.3	6.4	5.2
LATENCY TRIAL 2	MEAN	31.4	24.7	21.0
	S.D.	21.0	20.6	14.1
FAILED TO LEARN	N	0	0	0
	%	0.0%	0.0%	0.0%

**PASSIVE AVOIDANCE RETENTION**

<u>FEMALE RATS: DAY 2</u>	N	25	22	24
TRIALS TO CRITERION	MEAN	2.2	2.9	3.1
	S.D.	0.5	0.3	0.5
LATENCY TRIAL 1	MEAN	54.3	18.3	18.5
	S.D.	16.1	18.1	16.2

PASSIVE AVOIDANCE  
HISTORICAL CONTROL DATA  
CrI:CD@BR VAF/Plus@ RATS

CODE:	255	258	266
TYPE OF STUDY:	Seg. II/III	Seg. II/III	Seg. II/III (Behavior)
ROUTE:	Gavage	Gavage	Gavage
FINAL DRAFT REPORT MAIL DATE:	06/18/97	07/18/97	09/30/97
TESTING DATES:	11/06/96 - 11/18/96	12/04/96 - 12/16/96	02/25/97 - 03/10/97

**PASSIVE AVOIDANCE LEARNING**

<u>MALE RATS: DAY 1</u>	N	25	22	22
TRIALS TO CRITERION	MEAN	4.8	4.8	4.8
	S.D.	1.2	1.1	1.1
LATENCY TRIAL 1	MEAN	7.3	5.0	6.8
	S.D.	4.2	3.2	5.0
LATENCY TRIAL 2	MEAN	18.8	19.1	20.8
	S.D.	18.7	16.5	18.2
FAILED TO LEARN	N	0	0	0
	%	0.0%	0.0%	0.0%

**PASSIVE AVOIDANCE RETENTION**

<u>MALE RATS: DAY 2</u>	N	25	22	22
TRIALS TO CRITERION	MEAN	2.8	3.0	3.0
	S.D.	0.6	0.7	0.9
LATENCY TRIAL 1	MEAN	25.8	19.1	19.3
	S.D.	21.5	21.3	20.3

**PASSIVE AVOIDANCE LEARNING**

<u>FEMALE RATS: DAY 1</u>	N	25	22	23
TRIALS TO CRITERION	MEAN	4.8	4.8	5.3
	S.D.	1.6	1.1	1.3
LATENCY TRIAL 1	MEAN	8.6	7.7	6.0
	S.D.	10.2	4.4	3.3
LATENCY TRIAL 2	MEAN	21.9	19.6	13.1
	S.D.	17.3	16.0	10.2
FAILED TO LEARN	N	0	0	0
	%	0.0%	0.0%	0.0%

**PASSIVE AVOIDANCE RETENTION**

<u>FEMALE RATS: DAY 2</u>	N	25	22	23
TRIALS TO CRITERION	MEAN	2.7	3.0	3.1
	S.D.	0.5	0.6	0.6
LATENCY TRIAL 1	MEAN	26.8	19.4	20.0
	S.D.	24.9	20.8	17.4

PASSIVE AVOIDANCE  
HISTORICAL CONTROL DATA  
Crt:CD@BR VAF/Plus® RATS

CODE:	305
TYPE OF STUDY:	Comb. Pilot and
ROUTE:	Full Dev. and Repro.
FINAL DRAFT REPORT MAIL DATE:	06/23/98
TESTING DATES:	12/03/97 - 12/15/97

**PASSIVE AVOIDANCE LEARNING**

<u>MALE RATS: DAY 1</u>	N	21
TRIALS TO CRITERION	MEAN	4.7
	S.D.	1.0
LATENCY TRIAL 1	MEAN	6.4
	S.D.	5.5
LATENCY TRIAL 2	MEAN	21.9
	S.D.	16.4
FAILED TO LEARN	N	0
	%	0.0%

**PASSIVE AVOIDANCE RETENTION**

<u>MALE RATS: DAY 2</u>	N	21
TRIALS TO CRITERION	MEAN	2.9
	S.D.	0.3
LATENCY TRIAL 1	MEAN	13.6
	S.D.	13.5

**PASSIVE AVOIDANCE LEARNING**

<u>FEMALE RATS: DAY 1</u>	N	21
TRIALS TO CRITERION	MEAN	5.0
	S.D.	1.5
LATENCY TRIAL 1	MEAN	6.3
	S.D.	3.8
LATENCY TRIAL 2	MEAN	18.7
	S.D.	16.2
FAILED TO LEARN	N	0
	%	0.0%

**PASSIVE AVOIDANCE RETENTION**

<u>FEMALE RATS: DAY 2</u>	N	21
TRIALS TO CRITERION	MEAN	2.8
	S.D.	0.7
LATENCY TRIAL 1	MEAN	25.4
	S.D.	24.0

PASSIVE AVOIDANCE  
HISTORICAL CONTROL DATA  
Crl:CD@BR VAF/Plus@ RATS

CODE:  
TYPE OF STUDY:  
ROUTE:  
FINAL DRAFT REPORT MAIL DATE:  
TESTING DATES:

SUMMARY

10 STUDIES INCLUDED IN ANALYSIS

PASSIVE AVOIDANCE LEARNING		AVERAGE	MINIMUM	MAXIMUM
<u>MALE RATS: DAY 1</u>	N	23.2	21.0	25.0
TRIALS TO CRITERION	MEAN S.D.	4.8	4.3	5.4
LATENCY TRIAL 1	MEAN S.D.	7.6	5.0	11.7
LATENCY TRIAL 2	MEAN S.D.	25.5	18.8	35.0
FAILED TO LEARN	N %	0.1 0.0	0.0 0.0	1.0 0.0
PASSIVE AVOIDANCE RETENTION				
<u>MALE RATS: DAY 2</u>	N	23.1	21.0	25.0
TRIALS TO CRITERION	MEAN S.D.	2.8	2.1	3.1
LATENCY TRIAL 1	MEAN S.D.	27.9	13.6	56.9
PASSIVE AVOIDANCE LEARNING				
<u>FEMALE RATS: DAY 1</u>	N	23.1	21.0	25.0
TRIALS TO CRITERION	MEAN S.D.	4.8	4.4	5.3
LATENCY TRIAL 1	MEAN S.D.	6.7	5.0	8.6
LATENCY TRIAL 2	MEAN S.D.	24.2	13.1	35.9
FAILED TO LEARN	N %	0.1 0.0	0.0 0.0	1.0 0.0
PASSIVE AVOIDANCE RETENTION				
<u>FEMALE RATS: DAY 2</u>	N	23.0	21.0	25.0
TRIALS TO CRITERION	MEAN S.D.	2.9	2.2	3.2
LATENCY TRIAL 1	MEAN S.D.	27.1	18.3	54.3

APPENDIX 2  
WATERMAZE HISTORICAL CONTROL

WATERMAZE  
HISTORICAL CONTROL DATA  
Crl:CD@BR VAF/Plus@ RATS

CODE:	160.1	187	192
TYPE OF STUDY:	Seg. III	Seg. II/III	Seg. II/III
ROUTE:	Subcu.	Gavage	Gavage
FINAL DRAFT REPORT MAIL DATE:	11/24/95	07/23/96	08/30/96
TESTING DATES:	06/24/95 - 07/09/95	01/22/96 - 02/02/96	02/19/96-03/01/96

**WATERMAZE LEARNING**

<u>MALE RATS: DAY 1</u>	N	24	23	23
TOTAL TRIALS TO CRITERION	MEAN	8.8	8.9	9.5
	S.D.	2.3	2.7	3.2
ERRORS PER TRIAL	MEAN	0.35	0.44	0.34
	S.D.	0.15	0.24	0.13
LATENCY TRIAL 2	MEAN	11.4	18.0	12.4
	S.D.	8.7	14.7	7.0
FAILED TO LEARN	N	1	1	0
	%	4.2%	4.3%	0.0%

**WATERMAZE RETENTION**

<u>MALE RATS: DAY 2</u>	N	23	22	23
TOTAL TRIALS TO CRITERION	MEAN	5.8	6.6	6.1
	S.D.	1.7	2.2	1.7
ERRORS PER TRIAL	MEAN	0.06	0.09	0.12
	S.D.	0.11	0.13	0.14
LATENCY TRIAL 1	MEAN	6.7	7.1	9.6
	S.D.	2.8	3.8	7.8

**WATERMAZE**  
**HISTORICAL CONTROL DATA**  
**CrI:CD®BR VAF/Plus® RATS**

CODE:	234	242	251	255
TYPE OF STUDY:	Seg. II/III	Seg. II/III	Pre and Postnatal	Seg. II/III
ROUTE:	Gavage	IV	Dev.	Gavage
FINAL DRAFT REPORT MAIL DATE:	03/05/97	04/09/97	06/02/97	06/11/97
TESTING DATES:	08/26/96 - 09/06/96	09/03/96 - 09/08/96	06/15/96 - 06/28/96	12/22/96 - 01/03/97

**WATERMAZE LEARNING**

<u>MALE RATS: DAY 1</u>	N	24	24	32	25
TOTAL TRIALS TO CRITERION	MEAN	7.8	10.3	8.6	8.4
	S.D.	2.1	3.1	2.6	2.8
ERRORS PER TRIAL	MEAN	0.34	0.44	0.44	0.39
	S.D.	0.16	0.26	0.23	0.23
LATENCY TRIAL 2	MEAN	11.6	15.4	14.0	14.0
	S.D.	4.9	12.5	10.0	7.0
FAILED TO LEARN	N	0	3	1	1
	%	0.0%	12.5%	3.1%	4.0%

**WATERMAZE RETENTION**

<u>MALE RATS: DAY 2</u>	N	24	21	31	24
TOTAL TRIALS TO CRITERION	MEAN	6.3	6.1	7.1	6.6
	S.D.	2.1	1.7	2.6	2.6
ERRORS PER TRIAL	MEAN	0.14	0.10	0.15	0.12
	S.D.	0.17	0.14	0.18	0.16
LATENCY TRIAL 1	MEAN	11.7	10.6	10.9	9.5
	S.D.	8.0	7.2	7.2	6.2

**WATERMAZE**  
**HISTORICAL CONTROL DATA**  
**Crl:CD@BR VAF/Plus@ RATS**

CODE:	258	266	282	305
TYPE OF STUDY:	Seg. II/III	Seg. II/III	Pre and Post. Dev.	Comb. Pil. and Full
ROUTE:	Gavage	Gavage	Gavage	Dev. and Repro. Tox.
FINAL DRAFT REPORT MAIL DATE:	07/18/97	09/30/97	02/03/98	06/23/98
TESTING DATES:	01/20/97 - 01/31/97	04/14/97 - 04/25/97	07/28/97 - 08/08/97	01/19/98 - 01/30/98

**WATERMAZE LEARNING**

<u>MALE RATS: DAY 1</u>	N	22	23	22	22
TOTAL TRIALS TO CRITERION	MEAN	8.2	9.2	8.3	8.2
	S.D.	2.2	2.9	2.5	2.4
ERRORS PER TRIAL	MEAN	0.37	0.49	0.37	0.39
	S.D.	0.24	0.24	0.23	0.25
LATENCY TRIAL 2	MEAN	15.0	20.0	13.5	15.3
	S.D.	13.7	16.2	10.6	9.9
FAILED TO LEARN	N	0	1	1	1
	%	0.0%	4.3%	4.5%	4.5%

**WATERMAZE RETENTION**

<u>MALE RATS: DAY 2</u>	N	22	22	21	21
TOTAL TRIALS TO CRITERION	MEAN	7.3	7.5	5.9	6.5
	S.D.	3.1	3.0	1.6	2.3
ERRORS PER TRIAL	MEAN	0.13	0.17	0.14	0.09
	S.D.	0.14	0.16	0.30	0.11
LATENCY TRIAL 1	MEAN	8.7	10.4	12.6	7.7
	S.D.	4.5	6.5	12.6	3.0



**WATERMAZE**  
**HISTORICAL CONTROL DATA**  
 Crl:CD®BR VAF/Plus® RATS

CODE:  
 TYPE OF STUDY:  
 ROUTE:  
 FINAL DRAFT REPORT MAIL DATE:  
 TESTING DATES:

		TOTAL RATS      264			# STUDIES INCLUDED
WATERMAZE LEARNING		AVERAGE	MINIMUM	MAXIMUM	
<u>MALE RATS: DAY 1</u>	N	24	22	32	11
TOTAL TRIALS TO CRITERION	MEAN	8.7	7.8	10.3	11
	S.D.				
ERRORS PER TRIAL	MEAN	0.4	0.3	0.5	11
	S.D.				
LATENCY TRIAL 2	MEAN	14.6	11.4	20.0	11
	S.D.				
FAILED TO LEARN	N	1	0	3	11
	%	0	0	0	11
<b>WATERMAZE RETENTION</b>					
<u>MALE RATS: DAY 2</u>	N	23	21	31	11
TOTAL TRIALS TO CRITERION	MEAN	6.5	5.8	7.5	11
	S.D.				
ERRORS PER TRIAL	MEAN	0.1	0.1	0.2	11
	S.D.				
LATENCY TRIAL 1	MEAN	9.6	6.7	12.6	11
	S.D.				

**WATERMAZE**  
**HISTORICAL CONTROL DATA**  
**Cri:CD@BR VAF/Plus@ RATS**

CODE:	160.1	187	192
TYPE OF STUDY:	Seg. III	Seg. II/III	Seg. II/III
ROUTE:	Subcu.	Gavage	Gavage
FINAL DRAFT REPORT MAIL DATE:	11/24/95	07/23/96	08/30/96
TESTING DATES:	06/24/95 - 07/09/95	01/22/96 - 02/02/96	02/19/96-03/01/96

**FEMALE RATS**

**WATERMAZE LEARNING**

<u>FEMALE RATS: DAY 1</u>	N	23	23	22
TOTAL TRIALS TO CRITERION	MEAN	9.2	9.0	8.6
	S.D.	2.3	2.6	2.4
ERRORS PER TRIAL	MEAN	0.40	0.35	0.39
	S.D.	0.17	0.16	0.20
LATENCY TRIAL 2	MEAN	9.5	12.5	12.4
	S.D.	5.8	7.0	5.4
FAILED TO LEARN	N	1	1	0
	%	4.3%	4.3%	0.0%

**WATERMAZE RETENTION**

<u>FEMALE RATS: DAY 2</u>	N	22	22	22
TOTAL TRIALS TO CRITERION	MEAN	7.3	6.8	6.4
	S.D.	3.1	2.9	2.2
ERRORS PER TRIAL	MEAN	0.16	0.14	0.10
	S.D.	0.21	0.21	0.13
LATENCY TRIAL 1	MEAN	9.3	11.7	11.4
	S.D.	6.2	11.0	6.5

**WATERMAZE**  
**HISTORICAL CONTROL DATA**  
**Crl:CD®BR VAF/Plus® RATS**

CODE:	234	242	251	255
TYPE OF STUDY:	Seg. II/III	Seg. II/III	Pre and Postnatal	Seg. II/III
ROUTE:	Gavage	IV	Dev.	Gavage
FINAL DRAFT REPORT MAIL DATE:	03/05/97	04/09/97	06/02/97	06/11/97
TESTING DATES:	08/26/96 - 09/06/96	09/03/96 - 09/08/96	06/15/96 - 06/28/96	12/22/96 - 01/03/97

**FEMALE RATS**

**WATERMAZE LEARNING**

<u>FEMALE RATS: DAY 1</u>	N	22	24	33	25
TOTAL TRIALS TO CRITERION	MEAN	9.4	9.5	8.5	9.3
	S.D.	3.6	2.8	2.8	3.2
ERRORS PER TRIAL	MEAN	0.44	0.43	0.46	0.39
	S.D.	0.22	0.18	0.27	0.17
LATENCY TRIAL 2	MEAN	17.4	16.8	14.5	14.8
	S.D.	12.3	13.6	5.9	11.1
FAILED TO LEARN	N	1	1	2	2
	%	4.5%	4.2%	6.1%	8.0%

**WATERMAZE RETENTION**

<u>FEMALE RATS: DAY 2</u>	N	21	23	30	23
TOTAL TRIALS TO CRITERION	MEAN	8.5	7.8	7.1	6.9
	S.D.	3.4	3.3	2.0	2.7
ERRORS PER TRIAL	MEAN	0.18	0.22	0.20	0.21
	S.D.	0.19	0.23	0.22	0.21
LATENCY TRIAL 1	MEAN	9.7	15.0	10.6	13.8
	S.D.	6.3	12.8	5.2	8.8

**WATERMAZE**  
**HISTORICAL CONTROL DATA**  
**Crl:CD®BR VAF/Plus® RATS**

CODE:	258	266	282	305
TYPE OF STUDY:	Seg. II/III	Seg. II/III	Pre and Post. Dev.	Comb. Pil. and Full
ROUTE:	Gavage	Gavage	Gavage	Dev. and Repro. Tox.
FINAL DRAFT REPORT MAIL DATE:	07/18/97	09/30/97	02/03/98	06/23/98
TESTING DATES:	01/20/97 - 01/31/97	04/14/97 - 04/25/97	07/28/97 - 08/08/97	01/19/98 - 01/30/98

**FEMALE RATS**

**WATERMAZE LEARNING**

<u>FEMALE RATS: DAY 1</u>	N	22	23	22	21
TOTAL TRIALS TO CRITERION	MEAN	7.9	8.9	8.1	10.2
	S.D.	1.8	2.6	2.6	3.4
ERRORS PER TRIAL	MEAN	0.38	0.38	0.36	0.44
	S.D.	0.24	0.18	0.18	0.18
LATENCY TRIAL 2	MEAN	16.8	11.6	12.5	16.0
	S.D.	15.3	5.6	5.2	15.1
FAILED TO LEARN	N	0	0	0	3
	%	0.0%	0.0%	0.0%	14.3%

**WATERMAZE RETENTION**

<u>FEMALE RATS: DAY 2</u>	N	22	23	22	18
TOTAL TRIALS TO CRITERION	MEAN	7.4	7.5	7.3	6.7
	S.D.	2.8	2.2	2.7	2.3
ERRORS PER TRIAL	MEAN	0.14	0.25	0.13	0.15
	S.D.	0.15	0.24	0.14	0.18
LATENCY TRIAL 1	MEAN	11.1	10.2	12.5	12.0
	S.D.	7.0	5.8	7.6	10.0

**WATERMAZE**  
**HISTORICAL CONTROL DATA**  
**Cri:CD®BR VAF/Plus® RATS**

CODE:  
 TYPE OF STUDY:  
 ROUTE:  
 FINAL DRAFT REPORT MAIL DATE:  
 TESTING DATES:

FEMALE RATS		TOTAL RATS 260			# STUDIES INCLUDED
WATERMAZE LEARNING		AVERAGE	MINIMUM	MAXIMUM	
<u>FEMALE RATS: DAY 1</u>	N	24	21	33	11
TOTAL TRIALS TO CRITERION	MEAN S.D.	9.0	7.9	10.2	11
ERRORS PER TRIAL	MEAN S.D.	0.4	0.4	0.5	11
LATENCY TRIAL 2	MEAN S.D.	14.1	9.5	17.4	11
FAILED TO LEARN	N	1.0	0	3	11
	%	0.0	0.0	0.1	11
<b>WATERMAZE RETENTION</b>					
<u>FEMALE RATS: DAY 2</u>	N	23	18	30	11
TOTAL TRIALS TO CRITERION	MEAN S.D.	7.2	6.4	8.5	11
ERRORS PER TRIAL	MEAN S.D.	0.2	0.1	0.3	11
LATENCY TRIAL 1	MEAN S.D.	11.6	9.3	15.0	11

APPENDIX 3  
AUDITORY STARTLE HISTORICAL CONTROL

AUDITORY STARTLE HABITUATION  
HISTORICAL CONTROL DATA  
CrI:CD@BR VAF/Plus@ MALE RATS

CODE	131	134	160.1	228	251	290
DRAFT FINAL REPORT MAIL DATE	09/30/94	11/18/94	11/24/95	01/23/97	06/02/97	03/10/98
TYPE OF STUDY	Seg. III	Neurotox.	Seg. III	Pre and Postnatal Dev.	Pre and Postnatal Dev.	Neurotox.
TESTING DATES	01/24/94 - 03/08/94	03/23/94 - 05/05/94	05/08/95 - 06/24/95	07/12/96 - 07/17/96	05/01/96 - 05/07/96	10/23/97 - 12/06/97

AUDITORY STARTLE

RESPONSE MAGNITUDE

DAY 23 POSTPARTUM		(Day 22)		(Day 22)			
MALE RATS	N	24	20	24		33	20
BLOCK 1	MEAN	39.2	40.5	41.0		40.6	39.7
	S.D.	16.1	19.4	17.2		16.5	9.5
BLOCK 2	MEAN	30.6	35.1	35.7		32.3	38.6
	S.D.	15.1	16.8	18.5		16.5	10.4
BLOCK 3	MEAN	30.0	31.5	31.0		29.0	38.1
	S.D.	14.2	16.1	16.5		13.5	11.7
BLOCK 4	MEAN	31.0	30.8	30.9		28.6	43.5
	S.D.	17.8	17.9	17.0		14.6	11.6
BLOCK 5	MEAN	31.1	30.7	31.6		29.1	36.5
	S.D.	17.9	18.6	17.0		16.1	8.9
AVERAGE	MEAN	32.4	33.7	34.0		31.9	39.3
	S.D.	14.4	14.7	15.9		13.8	7.1

RESPONSE MAGNITUDE

DAY 60 POSTPARTUM

DAY 60 POSTPARTUM				(Day 61)			
MALE RATS	N	24	20	24	24		18
BLOCK 1	MEAN	87.9	108.1	183.4	218.9		36.9
	S.D.	47.7	60.6	115.8	142.9		7.5
BLOCK 2	MEAN	69.5	84.3	153.2	198.8		39.2
	S.D.	39.1	51.1	119.9	158.1		10.2
BLOCK 3	MEAN	64.1	64.7	125.3	143.5		34.2
	S.D.	38.2	34.1	84.8	93.6		7.5
BLOCK 4	MEAN	62.4	82.6	96.6	130.4		36.1
	S.D.	41.4	54.8	75.4	86.6		7.4
BLOCK 5	MEAN	64.7	77.3	104.8	114.0		36.3
	S.D.	39.4	49.6	67.6	60.2		10.0
AVERAGE	MEAN	69.7	83.4	132.7	161.1		36.5
	S.D.	37.7	47.7	84.2	97.8		6.5

AUDITORY STARTLE HABITUATION  
HISTORICAL CONTROL DATA  
Crl:CD®BR VAF/Plus® MALE RATS

CODE

DRAFT FINAL REPORT MAIL DATE

TYPE OF STUDY

SUMMARY

TESTING DATES

121 TOTAL MALE RATS

AUDITORY STARTLE

# STUDIES  
INCLUDED

RESPONSE MAGNITUDE

AVERAGE MINIMUM MAXIMUM IN ANALYSIS

DAY 23 POSTPARTUM

MALE RATS

N

BLOCK 1	MEAN	40.2	39.2	41.0	5
	S.D.				
BLOCK 2	MEAN	34.5	30.6	38.6	5
	S.D.				
BLOCK 3	MEAN	31.9	29.0	38.1	5
	S.D.				
BLOCK 4	MEAN	33.0	28.6	43.5	5
	S.D.				
BLOCK 5	MEAN	31.8	29.1	36.5	5
	S.D.				
AVERAGE	MEAN	34.3	31.9	39.3	5
	S.D.				

RESPONSE MAGNITUDE

DAY 60 POSTPARTUM

MALE RATS

N

BLOCK 1	MEAN	127.0	36.9	218.9	5
	S.D.				
BLOCK 2	MEAN	109.0	39.2	198.8	5
	S.D.				
BLOCK 3	MEAN	86.4	34.2	143.5	5
	S.D.				
BLOCK 4	MEAN	81.6	36.1	130.4	5
	S.D.				
BLOCK 5	MEAN	79.4	36.3	114.0	5
	S.D.				
AVERAGE	MEAN	96.7	36.5	161.1	5
	S.D.				



**AUDITORY STARTLE HABITUATION  
HISTORICAL CONTROL DATA  
CrI:CD®BR VAF/Plus FEMALE RATS**

CODE	131	134	160.1	228	251	290
DRAFT FINAL REPORT MAIL DATE	09/30/94	11/18/94	11/24/95	01/23/97	06/02/97	03/10/98
TYPE OF STUDY	Seg. III	Neurotox.	Seg. III	Pre and Postnatal Dev.	Pre and Postnatal Dev.	Neurotox.
TESTING DATES	01/24/94 - 03/08/94	03/23/94 - 05/05/94	05/08/95 - 06/24/95	07/12/96 - 07/17/96	05/01/96 - 05/07/96	10/23/97 - 12/06/97

**AUDITORY STARTLE**

**RESPONSE MAGNITUDE**

<b>DAY 23 POSTPARTUM</b>		(Day 22)		(Day 22)		
<b>FEMALE RATS</b>	<b>N</b>	24	19	23	33	20
BLOCK 1	MEAN	39.2	36.0	41.0	38.9	37.3
	S.D.	14.7	14.6	16.6	15.0	8.6
BLOCK 2	MEAN	30.8	29.2	36.4	32.0	37.1
	S.D.	14.3	14.8	20.2	14.4	11.8
BLOCK 3	MEAN	29.6	29.1	33.4	32.0	37.8
	S.D.	12.7	12.4	17.7	12.2	12.0
BLOCK 4	MEAN	29.0	31.8	34.1	30.0	36.9
	S.D.	12.9	16.1	18.8	13.3	13.7
BLOCK 5	MEAN	32.9	29.0	33.4	31.2	36.4
	S.D.	16.6	15.4	20.2	12.9	11.0
AVERAGE	MEAN	32.3	31.0	35.7	32.8	37.1
	S.D.	12.0	13.1	17.3	12.0	8.8

**RESPONSE MAGNITUDE**

<b>DAY 60 POSTPARTUM</b>					(Day 61)	
<b>FEMALE RATS</b>	<b>N</b>	24	19	23	25	20
BLOCK 1	MEAN	48.3	54.1	104.2	111.4	38.9
	S.D.	21.4	19.2	60.7	56.2	10.3
BLOCK 2	MEAN	47.2	39.7	89.8	101.8	37.3
	S.D.	22.9	13.8	57.9	63.9	11.0
BLOCK 3	MEAN	44.9	41.6	75.6	101.3	41.0
	S.D.	21.4	21.8	40.3	104.5	15.0
BLOCK 4	MEAN	41.9	39.4	66.5	69.7	39.6
	S.D.	18.4	11.3	40.0	49.0	13.7
BLOCK 5	MEAN	43.9	38.9	65.4	62.9	39.9
	S.D.	24.5	23.9	37.7	25.7	9.6
AVERAGE	MEAN	45.2	42.8	80.3	89.4	39.3
	S.D.	18.3	13.0	40.8	50.9	9.2

AUDITORY STARTLE HABITUATION  
HISTORICAL CONTROL DATA  
CrI:CD®BR VAF/Plus FEMALE RATS

CODE  
DRAFT FINAL REPORT MAIL DATE  
TYPE OF STUDY

SUMMARY

TESTING DATES

119 TOTAL FEMALE RATS

AUDITORY STARTLE

# STUDIES  
INCLUDED

RESPONSE MAGNITUDE

AVERAGE MINIMUM MAXIMUM IN ANALYSIS

DAY 23 POSTPARTUM

FEMALE RATS	N				
BLOCK 1	MEAN	38.5	36.0	41.0	5
	S.D.				
BLOCK 2	MEAN	33.1	29.2	37.1	5
	S.D.				
BLOCK 3	MEAN	32.4	29.1	37.8	5
	S.D.				
BLOCK 4	MEAN	32.4	29.0	36.9	5
	S.D.				
BLOCK 5	MEAN	32.6	29.0	36.4	5
	S.D.				
AVERAGE	MEAN	33.8	31.0	37.1	5
	S.D.				

RESPONSE MAGNITUDE

DAY 60 POSTPARTUM

FEMALE RATS	N			
BLOCK 1	MEAN	71.4	38.9	111.4
	S.D.			
BLOCK 2	MEAN	63.2	37.3	101.8
	S.D.			
BLOCK 3	MEAN	60.9	41.0	101.3
	S.D.			
BLOCK 4	MEAN	51.4	39.4	69.7
	S.D.			
BLOCK 5	MEAN	50.2	38.9	65.4
	S.D.			
AVERAGE	MEAN	59.4	39.3	89.4
	S.D.			

APPENDIX 4  
WATERMAZE POSITIVE CONTROL

# Detection of Prenatal Effects on Learning as a Function of Differential Criteria

ELIZABETH A. LOCHRY,<sup>1</sup> ALAN M. HOBERMAN AND MILDRED S. CHRISTIAN

*Argus Research Laboratories, Inc., Horsham, PA 19044*

LOCHRY, E. A., A. M. HOBERMAN AND M. S. CHRISTIAN. *Detection of prenatal effects on learning as a function of differential criteria.* NEUROBEHAV TOXICOL TERATOL 7(6) 697-701, 1985.—The role of appropriate criteria for detecting prenatal effects on learning was evaluated in CrI:COBS\*CD\*(SD)BR rats using a two-choice spatial discrimination escape paradigm. Alcohol was the prenatal treatment, as it has been reported to produce learning deficits in rats. The offspring of dams which consumed either a lab chow diet or isocaloric liquid diets containing 0%, 17.5% or 35% ethanol-derived calories during pregnancy were reared by surrogate control dams and tested for watermaze learning at 20 days postparturition. Learning performance and the required number of trials to the selected criterion were interrelated: (1) four or five consecutive errorless trials were too difficult, as high error rates occurred in all groups, including the control; (2) two consecutive errorless trials, or the number of trials before the first errorless trial, were too simple, as low error rates occurred in all groups, including the high dosage group; and (3) three consecutive errorless trials revealed significant dosage-dependent learning decrements in the alcohol-exposed groups. The results indicate that selection of the appropriate criterion in learning paradigms is critical for valid testing of prenatal effects.

Prenatal alcohol	Discriminated escape	Criterion	Rats	Watermaze learning	Liquid diets	Learning
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THE value of a given behavioral test lies in its ability to detect real differences between control and test populations. The role of selecting appropriate test criteria for detection of prenatal effects on postnatal development is recognized as a critical step in performing valid developmental toxicity evaluations.

The use of "behavioral/functional" tests as valid endpoints for demonstrating teratogenic effects in standardized developmental toxicity screens has been criticized as being of minimal value due to the apparent variability and/or unreliability of the data. The discrepant findings between and/or within laboratories have been attributed to multiple factors such as age, sex, handling, experience and even seasonal variations [5, 7, 10].

Confounding "ceiling" or "floor" effects [3] are insidious factors which frequently contribute to apparent inconsistencies in the behavioral data, particularly learning evaluations. These confounding effects occur when the test criterion is either too easily met or too difficult to attain, and as a result, the treatment effect is "masked." Since the detection of an effect can occur only when the experimental population demonstrates performance which is either better or worse than control performance, determination of the appropriate criterion is a critical component in the design of sensitive behavioral paradigms. If the criterion is too difficult to attain, group differences may be masked by the high error

rates which will occur in all groups, including the control. If the criterion is too easily met, the low error rate in all groups, including the highest dosage group, may also mask group differences.

The present study was conducted in order to identify the appropriate learning criterion to test 20-day-old CrI:COBS\*CD\*(SD)BR rats in a spatial discrimination watermaze paradigm, based on a level of control performance which would allow the test populations a sufficient performance range to demonstrate either a relative improvement or decrement.

Alcohol was chosen as the most reliable means of inducing learning deficiencies in the experimental groups, as this finding has been well documented in the literature for a variety of learning paradigms [2, 4, 6, 8, 11, 13-16]. The watermaze escape paradigm was chosen as the behavioral test of interest because it is frequently used as a learning evaluation in developmental toxicity safety evaluations submitted to regulatory agencies, particularly those of Japan.

## METHOD

### *Prenatal Treatment*

Parent animals were male (300 to 350 g) and female (200 to 225 g) CrI:COBS\*CD\*(SD)BR rats, obtained from

<sup>1</sup>Requests for reprints should be addressed to Elizabeth A. Lochry, Ph.D., Argus Research Laboratories, Inc., 905 Sheehy Drive, Horsham, PA 19044.

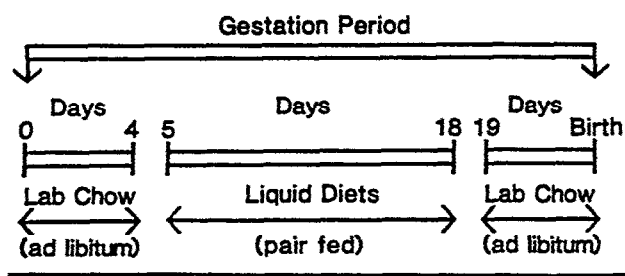


FIG. 1. Prenatal treatment regimen.

Charles River Breeding Laboratories, Inc., Kingston, New York. Animals were individually housed in wire-bottomed stainless steel cages suspended above absorbent paper liners and were maintained in a temperature and humidity controlled room on a 12-hr light-dark cycle with free access to feed [Certified Rodent Chow® #5002 (Ralston Purina)] and water (automatic watering system), except as noted.

Following an acclimation period of approximately two weeks, 150 virgin female rats were placed in cohabitation with male rats (one male/one female rat) for a maximum of eight days. Female rats with spermatozoa observed in vaginal smears, or a vaginal plug observed *in situ* or in the cage pan, were considered to be at day 0 of presumed gestation and assigned to individual housing. On day 18 of gestation, dams were transferred to opaque polycarbonate nesting boxes (Beta Chip® bedding) with wire lids. Male rats were used only as breeders and were removed from the study at the end of the cohabitation period.

A diagram of the prenatal treatment procedure is presented in Fig. 1. The period of exposure and the levels of alcohol used were selected on the basis of previous studies which had reported alcohol-induced learning deficiencies in rats [11, 13–15]. On days 5 through 18 of gestation, 14 female rats were given free access to a liquid diet containing 35% ethanol derived calories (EDC). Two other groups of 14 rats each were allowed access to either a 17.5% EDC or 0% EDC diet. The 17.5% and 0% EDC diets were isocaloric to the 35% EDC diet (1.3 Kcal/mL) supplying sucrose, in conjunction with, or instead of, ethanol. The compositions of the liquid diet have been described in detail previously [11]. Briefly, the diets contained chocolate Sustacal (Mead Johnson, Inc.), Vitamin Diet Fortification Mixture (ICN Nutritional Biochemicals), 95% ethanol and/or sucrose.

A pair-feeding procedure was used to control for daily caloric intake. Dams in the 35% EDC group were allowed free access to their diets, but dietary intake was restricted for dams in the remaining two groups. Each dam in the 17.5% EDC and the 0% EDC group was matched to a 35% EDC dam which had free access to its diet and was fed the amount consumed by this 35% EDC dam on a mL/kg/day basis. This procedure required that the 35% EDC dam within a yoked triad be at least one or two days more advanced in pregnancy than matched 17.5% EDC and 0% EDC dams. Thus, because the diets were isocaloric and were pair-fed on a body weight basis, each dam within a triad received the same number of calories for any given day of pregnancy, with only the dosage of alcohol varied. Diets were administered fresh daily at approximately 0900 hr. In order to obtain more precise control over pair-feeding, each dam was weighed again on days 5,

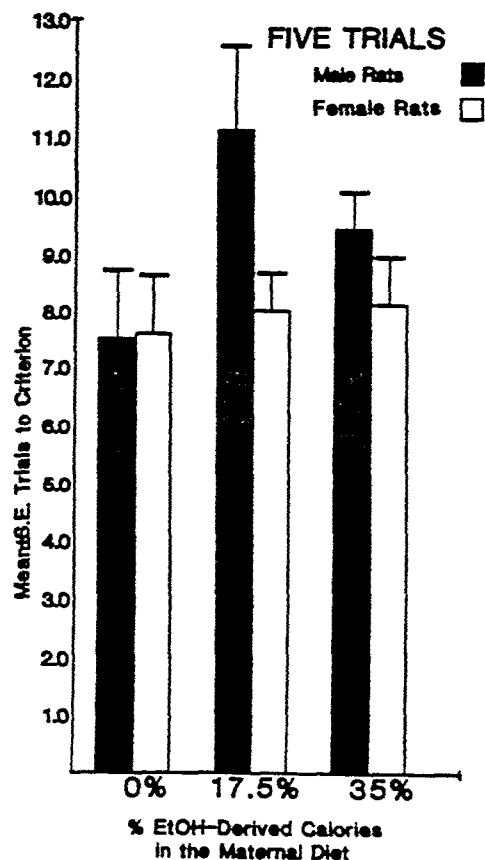


FIG. 2. The mean number of trials ( $\pm$  SE) to a learning criterion of five consecutive errorless trials, during the acquisition of a spatial discrimination watermaze test for offspring in the 0%, 17.5% and 35% EDC groups.

10, 15 and 18 of gestation; dams were also weighed on day 20 of gestation.

To assess any effect of the liquid diets, a fourth group of 14 dams was included which had free access to standard lab chow and water throughout gestation. This ad lib laboratory chow group (LC) was compared to the 0% EDC group by separate statistical analyses for each dependent measure, to assess any effect of restricted caloric intake due to the pair-feeding procedure.

In order to include a "fostering" procedure by which untreated "surrogate" dams reared the litters born to the LC and the 0%, 17.5% and 35% EDC groups, an additional group of 73 pregnant female rats was included. Each of these potential "surrogate" dams received free access to standard lab chow and water throughout gestation. "Surrogate" dams which delivered a litter up to 24 hours prior to the delivery of a LC, 0% EDC, 17.5% EDC or 35% EDC litter were used as "surrogate" dams. The biological offspring of the surrogate dams and the biological mothers of the LC, 0% EDC, 17.5% EDC and 35% EDC offspring were sacrificed.

From day 21 of gestation until delivery, the dams were checked three times daily for litters. Newborn pups were weighed and examined for gross external variations. Litters were culled to 10 pups each, and whenever possible, the litters were culled to five male and five female pups each.

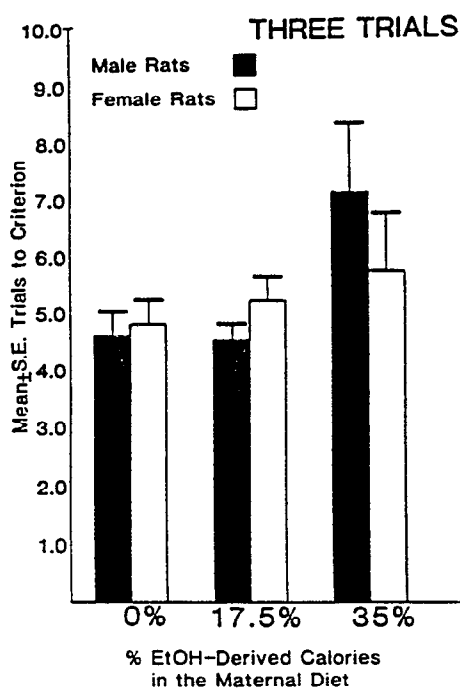


FIG. 3. The mean number of trials ( $\pm$ SE) to a learning criterion of three consecutive errorless trials, during the acquisition of a spatial discrimination watermaze test for offspring in the 0%, 17.5% and 35% EDC group.

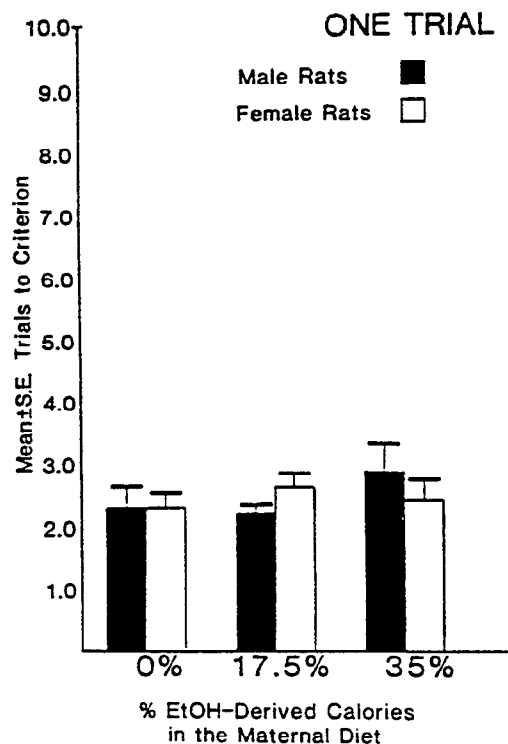


FIG. 4. The mean number of trials ( $\pm$ SE) to a learning criterion of one errorless trial, during the acquisition of a spatial discrimination watermaze test for offspring in the 0%, 17.5% and 35% EDC groups.

Body weights were recorded again on days 7, 14 and 20 postparturition.

#### BEHAVIORAL TESTING

##### Animals

A total of 10, 13, and 13 male pups and 10, 13 and 11 female pups from the 0%, 17.5% and 35% EDC groups, respectively, were evaluated on day 20 postparturition for learning performance in a water-filled T-maze. Pups were randomly selected from the various litters, such that no more than one male and one female pup were chosen from any one litter. At the time of testing, the selected animals were still housed in the common nesting box (with the dam and littermates). All pups were sacrificed (carbon dioxide) following completion of testing. At no time during the conduct of the behavioral test was the experimenter aware of the prenatal treatment condition of the animals.

##### Apparatus

An 18-gauge stainless steel T-maze was used to conduct the spatial discrimination water escape task. The concourse of the maze was 106.5 cm long, 15.5 cm wide and 20.5 cm high. The apparatus was approximately  $\frac{3}{4}$  filled with water. The water temperature was maintained at  $22 \pm 1^\circ\text{C}$ . Water temperature was monitored by a thermometer, which was attached to the floor of the apparatus. Each trial and inter-trial interval was timed with a stopwatch.

On the day of testing, each pup was removed from the

home cage, placed into a holding cage and transferred to the testing room. The pup was then placed into the water at the starting position (stem of the maze) facing away from the choice point. The pup was required to swim to one of the two goals in order to escape from the water. In order to control for "position preference" [12], the correct goal for each individual animal was the nonpreferred goal (the goal not initially entered on the first trial). Thus, if the rat initially entered the right goal on the first trial, the left goal was the correct goal for that animal. The rat was required to touch the far wall of the correct goal, in order to be removed from the water. Escape ramps were not used for this test, as the learning paradigm was strictly a spatial, rather than a visual, discrimination problem, and an escape ramp would have served as a visual cue at the choice point. Following a correct response, the animal was removed from the maze and placed into a holding cage. An intertrial interval of approximately 30 seconds separated each trial. Rats failing to make a correct goal choice within 30 seconds on any given trial were guided to the correct goal, removed from the maze and assigned a latency of 30 seconds. Each rat was required to reach a criterion of five consecutive errorless trials to terminate the testing session. A maximum of 20 trials was given. All rats were sacrificed after completion of testing.

Animals were tested using the most difficult criterion (five consecutive errorless trials), and the data were then scored for the number of trials required to satisfy a learning criterion of either: (1) five consecutive errorless trials; (2) four consecutive errorless trials; (3) three consecutive errorless

trials; (4) two consecutive errorless trials; or (5) one trial without errors. The latency for each trial (time in seconds to swim from the start position to the correct goal) and the number of errors (incorrect turns in the maze) were recorded. Additionally, groups were compared for the number of errors per trial and the latencies on the first and second trials.

## RESULTS

### *Offspring Viability and Maternal Parameters*

Dams in the 35% EDC group consumed an average ( $\pm$ SE) of 13.85 ( $\pm$ 0.35) g ethanol per kg body weight per day. Dams in the pair-fed 17.5% EDC group consumed an average of 6.8 ( $\pm$ 1.0) g/kg/day. No clinical signs associated with withdrawal reactions were noted following termination of access to the alcohol containing liquid diets.

Of the 129 female rats which were mated (56 assigned to the three liquid diet or LC groups and 73 potential "surrogate" dams), eight were not pregnant (3, 1 and 1 female rats in the 0%, 17.5% and 35% EDC groups, respectively, and 3 potential "surrogate" dams).

A one-way Analysis of Variance (ANOVA) with Alcohol (0%, 17.5% and 35% EDC) as a between-subjects factor was conducted on percent maternal weight gain during pregnancy, duration of gestation, litter size and percentage of surviving pups per litter. Trend decompositions were performed subsequent to the overall analysis, when appropriate [9,19]. Separate analyses were conducted to evaluate nutritional effects by comparing the 0% EDC and lab chow control groups. This latter group is not on the same variable dimension as the liquid diet groups, and its inclusion in the overall analyses would have precluded decomposition into trend components.

Average percent maternal weight gain during gestation was equivalent for the three liquid diet groups: a main effect of Alcohol was not significant ( $p>0.05$ ). However, as typically seen with alcohol administration via liquid diets, maternal weight gain for the 0% EDC group was less than that of the LC group. Significant differences occurred between the 0% EDC dams (which had restricted caloric intake) and the LC dams (which had free access to lab chow and water),  $F(1,23)=4.81$ ,  $p\leq 0.05$ , indicating that the caloric restriction resulting from the pair-feeding procedure affected maternal body weight gain during gestation for dams in each of the liquid diet groups.

Duration of gestation, delivered litter size and pup survival per litter were not affected by either alcohol consumption or caloric restriction. Differences among the three liquid diet groups were neither significant ( $p>0.05$ ) nor dosage-dependent. The statistical analyses for nutritional effects between the 0% EDC and LC groups were not significant ( $p>0.05$ ).

### *Offspring Body Weight and Behavioral Parameters*

Offspring body weight and behavioral parameters were analyzed by a  $3\times 2$  analysis of variance (ANOVA) using Alcohol (0%, 17.5%, 35% EDC) and Gender (Male, Female) as between-subjects factors. The litter was the unit of analysis for these offspring parameters [1, 7, 17, 18]. Trend decompositions were performed subsequent to the ANOVAs, when appropriate [9,19]. As previously stated for the maternal parameters, separate analyses were conducted

for all comparisons of the 0% EDC and LC control groups, to allow conduct of the trend decompositions for the liquid diet groups.

*Offspring body weight.* Maternal alcohol consumption during pregnancy resulted in dosage-dependent decreases in average pup body weight for days 7, 14 and 20 postparturition. A significant linear trend was present for average litter weight for days 7,  $F(1,68)=5.15$ ,  $p\leq 0.05$ , 14,  $F(1,68)=14.17$ ,  $p\leq 0.01$ , and 20,  $F(1,68)=12.13$ ,  $p\leq 0.01$ , postparturition. Male rats weighed more than female rats for each of the days that body weight was monitored, however, differences were significant only for birth weight,  $F(1,68)=8.53$ ,  $p\leq 0.01$ . The interaction of Alcohol with Gender was not significant for any of the analyses. The statistical analyses for nutritional effects, comparing the 0% EDC and the LC control groups, failed to indicate any significant differences between these two groups ( $F<1$ ) for average pup body weight per litter.

*Criterion of four or five consecutive errorless trials.* No significant differences due to Alcohol, Gender or the interaction of these factors were apparent between the liquid diet groups for the number of trials required to satisfy a learning criterion of either four or five consecutive errorless trials (Fig. 2). For both of these learning criteria, a high error rate was apparent for all groups, including the control group. Significant differences did not occur for either the total number of errors made during the test session or the average number of errors per trial.

*Criterion of three consecutive errorless trials.* As presented in Fig. 3, the alcohol-exposed offspring required a significantly greater number of trials to meet the learning criterion of three consecutive errorless trials,  $F(2,64)=3.31$ ,  $p\leq 0.05$ . The analysis revealed a significant linear trend,  $F(1,64)=5.28$ ,  $p\leq 0.01$ , indicating that the number of trials required to meet the three consecutive errorless trial learning criterion was directly related to the level of *in utero* alcohol exposure. Significant effects were not apparent when the groups were compared for the total number of errors per session or the average number of errors per trial, indicating that the overall error rate during this task was not as sensitive an indicator of learning as the pattern of errors occurring across trials.

*Criterion of one errorless trial or two consecutive errorless trials.* No significant differences due to Alcohol, Gender or the interaction of these factors were apparent between the liquid diet groups for the number of trials required to satisfy a learning criterion of either one errorless trial (Fig. 4) or two consecutive errorless trials. For both of these learning criteria, a low error rate was evident for all groups, including the high dosage group. Significant differences did not occur for the total number of errors made during the test session or the average number of errors per trial.

For all of the learning criteria evaluated, male rats did not perform differently from female rats. Gender and the interaction of Gender with Alcohol were not significant factors ( $p>0.05$ ). The nutritional comparisons between the 0% EDC and the LC controls failed to demonstrate any significant differences between these two groups ( $F<1$ ) for any of the different learning criteria.

## DISCUSSION

The results of the current investigation indicate that when treatment effects exist, the extent to which these effects can be detected by functional evaluations may depend on the appropriateness of the test criterion. Moreover, the appro-

priateness of the test criterion is determined by the performance level of the control group.

In the spatial discrimination watermaze task used, the number of trials prior to attaining a learning criterion of four or five consecutive errorless trials was too difficult. A classic "floor effect" occurred and masked group differences; a high error rate occurred for all the groups, including the control group. The number of trials prior to attaining a learning criterion of the first errorless trial or two consecutive errorless trials was too lenient. A classic "ceiling effect" occurred masking group differences [3]; a low error rate occurred for all groups, including the 35% EDC group. The use of a learning criterion of three consecutive errorless trials was most effective in distinguishing the offspring exposed to alcohol *in utero* from the controls. As this criterion elicited a moderate error rate from the control offspring, it

was determined to be the most appropriate criterion for this learning paradigm for rats of this age and strain.

These results indicate that selection of the appropriate criterion in learning paradigms is critical for valid postnatal testing of prenatal effects. The ideal criterion for a learning evaluation is one which requires a moderate level of challenge for the control population, and allows the experimental groups to demonstrate relative improvements or decrements in performance. These findings also demonstrate that arbitrarily setting a test criterion on the assumption that any given test criterion will reflect group differences, does not necessarily provide a sensitive test. The importance of knowing the normal range of the control population in determining the ideal test criterion for sensitive behavioral/functional evaluations cannot be overstated.

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APPENDIX 5  
MOTOR ACTIVITY POSITIVE CONTROL #1

**Validation of a Functional Observational Battery  
and Motor Activity Measure  
Using Positive Test Substances**

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## ABSTRACT

The EPA guidelines for neurotoxicity assessments are expected to be finalized by January 1991. These assessments are expected to include a Functional Observational Battery (FOB) and motor activity measurement. To validate the FOB a single dosage of p,p' DDT (75 mg/kg, p.o.) and physostigmine monosalicylate (0.75 mg/kg, IP), and repeated dosages of acrylamide (50 mg/kg, IP, every other day) were used. The test substances and their vehicles were administered to separate groups of adult male (10) and female (10) rats (Crl:CD@BR VAF/Plus®). Each test substance produced different patterns of effects. The rats administered DDT did not differ from controls 1 hour after administration; after 5 hours, tremors were evident throughout the body, reactivity to a visual stimulus was increased, and grip strength and foot splay were decreased. Physostigmine produced increased salivation, miosis, tremors in the limbs and changes in gait at 15 minutes after administration but not at 2 hours. Acrylamide produced an increase in foot splay and changes in gait following eight dosages. To validate the motor activity assessment, groups of seven male and female rats were administered chlorpromazine (2.0 mg/kg, IP) and its vehicle 70 minutes prior to testing, or amphetamine (1.0 mg/kg, IP) and its vehicle immediately before the test session. Motor activity was assessed for a 2-hour period. All groups diminished their activity throughout the session, but the habituation was less evident in the group given amphetamine. In contrast, the groups given chlorpromazine decreased their activity at a faster rate than the two control groups.

## FUNCTIONAL OBSERVATIONAL BATTERY

The test items were principally derived from published protocols (Haggerty, G.C., J. Am. Coll. Toxicol. 1989, 8, 53-69; Moser, V. C., J. Am. Coll. Toxicol. 1989, 8, 85-93; Moser, V. C. et al., Fundam. Appl. Toxicol., 1988, 11, 189-206; O'Donoghue, J. L., J. Am. Coll. Toxicol., 1989, 8, 97-115) and were based on the current EPA guideline (40 CFR 798.6050) and proposed revisions. The grip-strength device was patterned after the device described by Mattson et al. (Fundam. Appl. Toxicol., 1986, 6, 175-181), a modification of the apparatus described by Meyer et al. (Neurobehav. Toxicol., 1979, 1, 233-236). The tests were conducted under GLP guidelines. Statistical analyses for dosage effects were performed separately for each item at each test session and for each sex. Items with descriptive or binary elements were first analyzed as 2 x n contingency tables, and significant overall results were followed by 2 x 2 comparisons for each element. Items with graded scores and count data were evaluated using the Kruskal-Wallis procedure. For the tables presented here, the data for both sexes were tabulated together for the items with those data types. Items with interval data were analyzed by ANOVA or the Kruskal-Wallis procedure depending upon the outcome of Bartlett's test for equality of variance. The data for males and females are displayed separately for those items.

## **DDT (75 mg/kg)**

Statistically significant differences between dosage groups were observed for the following test items five hours after dosage.

- Home Cage Behavior
- Involuntary Behavior at the Home Cage and in the Open Field
- Reaction to a Visual Stimulus
- Grip Strength
- Foot Splay

GROUP DOSAGE (MG/KG/DAY) <sup>a</sup> - DDT		I 0 (VEHICLE CONTROL)			II 75		
TEST		PRE- DOSAGE	POST-DOSAGE		PRE- DOSAGE	POST-DOSAGE	
			1HR	5HR		1HR	5HR
RATS TESTED	N	20	20	20	20	20	20
HOME CAGE BEHAVIOR							
Apparently sleeping	N	5	4	6	6	1	1
Awake, immobile, normal posture	N	8	9	12	8	10	1**
Engaged in normal behavior	N	7	7	2	6	9	0
Immobile, unusual behavior	N	0	0	0	0	0	0
Engaged in unusual behavior	N	0	0	0	0	0	16**
INVOLUNTARY BEHAVIOR <sup>b</sup>							
Not apparent	N	20	20	20	20	20	2**
Stereotyped behavior pattern	N	0	0	0	0	0	1
Bizarre behavior	N	0	0	0	0	0	0
Tremors in limbs	N	0	0	0	0	0	0
Whole body tremors or spasms	N	0	0	0	0	0	17**
Unusual posture	N	0	0	0	0	0	0
Tonic-clonic seizure	N	0	0	0	0	0	0
REACTION TO REMOVAL							
(1) Sits quietly	N	10	12	11	11	10	14
(2) Vocalization without resistance	N	8	6	7	4	6	2
(3) Runs around cage	N	2	2	2	4	4	3
(4) Freezes and rears, follows hand	N	0	0	0	1	0	0
(5) Tail and throat rattles	N	0	0	0	0	0	1
MEDIAN SCORE		1.5	1.0	1.0	1.0	1.5	1.0
REACTION TO HANDLING							
(1) Quiet with no resistance	N	14	16	16	11	15	15
(2) Vocalization without resistance	N	2	3	3	3	1	1
(3) Tense and rigid	N	3	0	1	3	1	0
(4) Squirming and twisting	N	1	1	0	3	3	4
MEDIAN SCORE		1.0	1.0	1.0	1.0	1.0	1.0
PALPEBRAL CLOSURE							
(1) Eyelids wide open	N	20	20	20	20	20	20
(2) Eyelids slightly drooping	N	0	0	0	0	0	0
(3) Eyelids drooping, half-closed	N	0	0	0	0	0	0
(4) Eyelids completely shut	N	0	0	0	0	0	0
MEDIAN SCORE		1.0	1.0	1.0	1.0	1.0	1.0
PROMINENCE OF EYE							
Normal	N	20	20	20	20	20	20
Exophthalmos	N	0	0	0	0	0	0
Enophthalmos	N	0	0	0	0	0	0

GROUP		I			II		
DOSAGE (MG/KG/DAY) <sup>a</sup> - DDT		0(VEHICLE CONTROL)			75		
TEST		PRE-	POST-DOSAGE		PRE-	POST-DOSAGE	
		DOSAGE	1HR	5HR	DOSAGE	1HR	5HR
LACRIMATION							
(1) No excessive lacrimation	N	20	20	20	20	20	20
(2) Slight	N	0	0	0	0	0	0
(3) Severe	N	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0
SALIVATION							
(1) No excessive salivation	N	20	19	20	20	20	20
(2) Wet margin along submaxillary area	N	0	0	0	0	0	0
(3) Severe	N	0	1	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0
PILOERECTION <sup>c</sup>	N	0	0	0	0	0	0
ABNORMAL RESPIRATION <sup>c</sup>	N	0	0	0	0	0	0
APPEARANCE							
(1) Clean and groomed	N	20	20	20	20	20	20
(2) Unkempt	N	0	0	0	0	0	0
(3) Stained by urine and feces	N	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0
AROUSAL LEVEL							
(1) Very low (stuporous)	N	0	0	0	0	0	0
(2) Low (some exploratory movement)	N	0	0	3	0	0	0
(3) Apparently normal	N	20	20	17	20	20	18
(4) High (sudden darting and freezing)	N	0	0	0	0	0	2
(5) Very high (running, vocalization)	N	0	0	0	0	0	0
	MEDIAN SCORE	3.0	3.0	3.0	3.0	3.0	3.0
DEGREE OF MOTILITY							
(1) Apparently normal	N	20	20	20	20	20	11
(2) Moves with difficulty	N	0	0	0	0	0	9
(3) Unable to move	N	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0#
GAIT							
Normal	N	20	20	20	20	20	13#
Ataxic (swaying or lurching)	N	0	0	0	0	0	0
Exaggerated movements in limbs	N	0	0	0	0	0	7#
Tip-toe walk	N	0	0	0	0	0	0

GROUP		I			II		
DOSAGE (MG/KG/DAY) <sup>a</sup> - DDT		0 (VEHICLE CONTROL)			75		
TEST		PRE-DOSAGE	POST-DOSAGE 1HR	POST-DOSAGE 5HR	PRE-DOSAGE	POST-DOSAGE 1HR	POST-DOSAGE 5HR
INVOLUNTARY BEHAVIOR <sup>d</sup>							
None apparent	N	20	20	20	20	20	2**
Stereotyped behavior pattern	N	0	0	0	0	0	0
Bizarre behavior	N	0	0	0	0	0	0
Tremor in limbs	N	0	0	0	0	0	1
Whole body tremors or spasms	N	0	0	0	0	0	17**
Unusual posture	N	0	0	0	0	0	0
Tonic-clonic seizure	N	0	0	0	0	0	0
FECAL BOLUSES	MEAN	0.4	0.7	0.6	1.6	0.9	1.7
	± S.D.	± 0.8	± 1.3	± 1.2	± 2.3	± 1.3	± 1.9#
URINE POOLS	MEAN	1.0	1.0	0.5	1.0	0.9	1.0
	± S.D.	± 0.9	± 0.9	± 0.5	± 0.8	± 1.0	± 1.0
REACTION TO VISUAL STIMULUS							
(1) No reaction	N	3	5	7	5	2	3
(2) Moves toward or away from stimulus	N	17	15	12	13	18	6
(3) Startle response or freezing	N	0	0	1	2	0	10
(4) More energetic response	N	0	0	0	0	0	1
(5) Attacks or bites probe	N	0	0	0	0	0	0
	MEDIAN SCORE	2.0	2.0	2.0	2.0	2.0	3.0*
REACTION TO TACTILE STIMULUS							
(1) No reaction	N	9	7	13	10	13	11
(2) Moves toward or away from stimulus	N	4	3	2	7	4	5
(3) Startle response or freezing	N	7	10	5	3	3	4
(4) More energetic response	N	0	0	0	0	0	0
(5) Attacks or bites probe	N	0	0	0	0	0	0
	MEDIAN SCORE	1.5	2.5	1.0	1.5	1.0#	1.0
REACTION TO AUDITORY STIMULUS							
(1) No reaction	N	1	2	0	0	1	0
(2) Moves toward or away from response	N	0	1	0	2	1	0
(3) Startle response or freezing	N	18	15	19	18	18	16
(4) More energetic response	N	1	2	1	0	0	4
(5) Jumps at or away from the noise	N	0	0	0	0	0	0
	MEDIAN SCORE	3.0	3.0	3.0	3.0	3.0	3.0
REACTION TO TAIL PINCH							
(1) No reaction	N	11	11	14	6	7	9
(2) Moves toward or away from stimulus	N	5	7	5	8	8	10
(3) Startle response or freezing	N	3	2	1	6	5	1
(4) More energetic response	N	1	0	0	0	0	0
(5) Attacks or bites probe	N	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	2.0	2.0	2.0
PUPILLARY RESPONSE <sup>c</sup>	N	19	19	20	20	20	20



GROUP		I			II		
DOSAGE (MG/KG/DAY) <sup>a</sup> - DDT		O(VEHICLE CONTROL)			75		
TEST		PRE-	POST-DOSAGE		PRE-	POST-DOSAGE	
		DOSAGE	1HR	5HR	DOSAGE	1HR	5HR
AIR RIGHTING REACTION							
(1) Lands with all feet on the ground	N	19	20	19	20	20	16
(2) Uncoordinated landing	N	1	0	1	0	0	4
(3) Lands on back	N	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0
VISUAL PLACING REACTION							
(1) Early extension of forelimbs	N	20	20	20	20	20	20
(2) Extension follows initial contact	N	0	0	0	0	0	0
(3) No extension after contact	N	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0
MALES:	N	10	10	10	10	10	10
FORELIMB GRIP STRENGTH (MAXIMUM g)	MEAN	911	908	782	900	880	505
	± S.D.	± 156	± 114	± 113	± 113	± 155	± 219**
HINDLIMB GRIP STRENGTH (MAXIMUM g)	MEAN	913	938	884	844	944	804
	± S.D.	± 92	± 88	± 163	± 150	± 104	± 164
FOOT SPLAY MEASURE (AVERAGE cm)	MEAN	10.1	8.7	8.9	9.1	8.1	6.6
	± S.D.	± 3.2	± 2.3	± 1.4	± 2.3	± 2.1	± 0.6**
BODY WEIGHT (g)	MEAN	522	558		551	558	
	± S.D.	± 77	± 74		± 78	± 77	
FEMALES:	N	10	10	10	10	10	10
FORELIMB GRIP STRENGTH (MAXIMUM g)	MEAN	793	732	646	823	792	590
	± S.D.	± 163	± 180	± 155	± 154	± 213	± 151
HINDLIMB GRIP STRENGTH (MAXIMUM g)	MEAN	634	689	768	687	659	627
	± S.D.	± 153	± 169	± 108	± 94	± 188	± 162*
FOOT SPLAY MEASURE (AVERAGE cm)	MEAN	7.4	7.5	7.3	7.7	6.7	5.7
	± S.D.	± 2.2	± 1.6	± 1.4	± 1.7	± 1.6	± 1.2*
BODY WEIGHT (g)	MEAN	276	279		278	289	
	± S.D.	± 23	± 20		± 25	± 20	

HR = HOURS

(n) = Score assigned to graded test items.

- Dosage occurred once, on day 1 of the study.
- Observation made at the home cage.
- Number of rats showing a response.
- Observation made in the open field.

\* Significantly different from the vehicle control group value ( $P \leq 0.05$ ). For the items showing the combined data from both males and females, the differences were significant for both sexes.

\*\* Significantly different from the vehicle control group value ( $P \leq 0.01$ ). For the items showing the combined data from both males and females, the differences were significant for both sexes.

# Significantly different from the vehicle control group value ( $P \leq 0.05$ ) for one, but not both sexes (for the items showing the combined data from both males and females).

## **Physostigmine (0.75 mg/kg)**

Statistically significant differences between dosage groups were observed for the following test items 15 minutes after dosage.

- Home Cage Behavior
- Involuntary Behavior at the Home Cage and in the Open Field
- Reaction to Removal
- Salivation
- Arousal Level
- Degree of Motility
- Gait
- Reaction to Tail Pinch
- Pupillary Response

GROUP		I			II		
DOSAGE (MG/KG/DAY) <sup>a</sup> - PHYSOSTIGMINE		0 (VEHICLE CONTROL)			0.75		
TEST		PRE-	POST-DOSAGE		PRE-	POST-DOSAGE	
		DOSAGE	15M	2HR	DOSAGE	15M	2HR
RATS TESTED	N	20	20	20	20	20	20
HOME CAGE BEHAVIOR							
Apparently sleeping	N	6	0	11	3	0	3
Awake, immobile, normal posture	N	9	12	6	12	6	13
Engaged in normal behavior	N	5	8	3	5	0	4
Immobile, unusual behavior	N	0	0	0	0	0	0
Engaged in unusual behavior	N	0	0	0	0	14**	0
INVOLUNTARY BEHAVIOR <sup>b</sup>							
Not apparent	N	20	20	20	20	4**	20
Stereotyped behavior pattern	N	0	0	0	0	0	0
Bizarre behavior	N	0	0	0	0	0	0
Tremors in limbs	N	0	0	0	0	16**	0
Whole body tremors or spasms	N	0	0	0	0	0	0
Unusual posture	N	0	0	0	0	0	0
Tonic-clonic seizure	N	0	0	0	0	0	0
REACTION TO REMOVAL							
(1) Sits quietly	N	13	10	16	12	20	13
(2) Vocalization without resistance	N	2	5	2	4	0	6
(3) Runs around cage	N	5	4	2	3	0	1
(4) Freezes and rears, follows hand	N	0	1	0	1	0	0
(5) Tail and throat rattles	N	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.5	1.0	1.0	1.0*	1.0
REACTION TO HANDLING							
(1) Quiet with no resistance	N	11	15	19	11	19	18
(2) Vocalization without resistance	N	2	0	0	2	0	1
(3) Tense and rigid	N	2	1	1	1	0	0
(4) Squirming and twisting	N	5	4	0	6	1	1
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0
PALPEBRAL CLOSURE							
(1) Eyelids wide open	N	20	20	20	20	20	20
(2) Eyelids slightly drooping	N	0	0	0	0	0	0
(3) Eyelids drooping, half-closed	N	0	0	0	0	0	0
(4) Eyelids completely shut	N	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0
PROMINENCE OF EYE							
Normal	N	20	20	20	20	20	20
Exophthalmos	N	0	0	0	0	0	0
Enophthalmos	N	0	0	0	0	0	0

GROUP		I			II		
DOSAGE (MG/KG/DAY) <sup>a</sup> - PHYSOSTIGMINE		0 (VEHICLE CONTROL)			0.75		
TEST		PRE-DOSAGE	POST-DOSAGE 15M	2HR	PRE-DOSAGE	POST-DOSAGE 15M	2HR
LACRIMATION							
(1) No excessive lacrimation	N	20	20	20	20	20	20
(2) Slight	N	0	0	0	0	0	0
(3) Severe	N	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0
SALIVATION							
(1) No excessive salivation	N	20	20	19	20	11	19
(2) Wet margin along submaxillary area	N	0	0	1	0	3	1
(3) Severe	N	0	0	0	0	6	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0*	1.0
PILOERECTION <sup>c</sup>	N	0	0	0	0	0	0
ABNORMAL RESPIRATION <sup>c</sup>	N	0	0	0	0	0	0
APPEARANCE							
(1) Clean and groomed	N	20	20	20	20	20	20
(2) Unkempt	N	0	0	0	0	0	0
(3) Stained by urine and feces	N	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0
AROUSAL LEVEL							
(1) Very low (stuporous)	N	0	0	0	0	0	0
(2) Low (some exploratory movement)	N	0	0	0	0	6	0
(3) Apparently normal	N	20	20	20	20	14	20
(4) High (sudden darting and freezing)	N	0	0	0	0	0	0
(5) Very high (running, vocalization)	N	0	0	0	0	0	0
	MEDIAN SCORE	3.0	3.0	3.0	3.0	3.0#	3.0
DEGREE OF MOTILITY							
(1) Apparently normal	N	20	20	20	20	4	20
(2) Moves with difficulty	N	0	0	0	0	16	0
(3) Unable to move	N	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	2.0**	1.0
GAIT							
Normal	N	20	20	20	19	3**	20
Ataxic (swaying or lurching)	N	0	0	0	0	0	0
Exaggerated movements in limbs	N	0	0	0	0	17**	0
Tip-toe walk	N	0	0	0	1	0	0

GROUP		I			II		
DOSAGE (MG/KG/DAY) <sup>a</sup> - PHYSOSTIGMINE		0(VEHICLE CONTROL)			0.75		
TEST		PRE-	POST-DOSAGE		PRE-	POST-DOSAGE	
		DOSAGE	15M	2HR	DOSAGE	15M	2HR
INVOLUNTARY BEHAVIOR <sup>d</sup>							
None apparent	N	20	20	20	20	2**	20
Stereotyped behavior pattern	N	0	0	0	0	0	0
Bizarre behavior	N	0	0	0	0	0	0
Tremors in limbs	N	0	0	0	0	18**	0
Whole body tremors or spasms	N	0	0	0	0	0	0
Unusual posture	N	0	0	0	0	0	0
Tonic-clonic seizure	N	0	0	0	0	0	0
FECAL BOLUSES	MEAN	0.5	0.7	1.2	1.1	1.0	0.8
	± S.D.	± 0.9	± 1.4	± 1.3	± 1.0#	± 0.9	± 0.9
URINE POOLS	MEAN	1.0	0.9	0.7	0.8	1.0	0.6
	± S.D.	± 0.9	± 1.0	± 0.8	± 0.9	± 1.0	± 0.7
REACTION TO VISUAL STIMULUS							
(1) No reaction	N	6	8	8	2	7	8
(2) Moves towards or away from stimulus	N	14	10	12	18	13	9
(3) Startle response or freezing	N	0	2	0	0	0	3
(4) More energetic response	N	0	0	0	0	0	0
(5) Attacks or bites probe	N	0	0	0	0	0	0
	MEDIAN SCORE	2.0	2.0	2.0	2.0	2.0	2.0
REACTION TO TACTILE STIMULUS							
(1) No reaction	N	16	12	17	13	18	15
(2) Moves towards or away from stimulus	N	4	6	2	6	0	4
(3) Startle response or freezing	N	0	2	1	1	2	1
(4) More energetic response	N	0	0	0	0	0	0
(5) Attacks or bites probe	N	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0
REACTION TO AUDITORY STIMULUS							
(1) No reaction	N	1	1	2	0	3	1
(2) Moves toward or away from stimulus	N	2	0	1	1	0	1
(3) Startle response or freezing	N	16	19	17	19	12	18
(4) More energetic response	N	0	0	0	0	5	0
(5) Jumps at or away from the noise	N	0	0	0	0	0	0
	MEDIAN SCORE	3.0	3.0	3.0	3.0	3.0	3.0
REACTION TO TAIL PINCH							
(1) No reaction	N	8	6	9	8	18	10
(2) Moves towards or away from stimulus	N	6	9	10	6	0	5
(3) Startle response or freezing	N	4	4	1	5	2	5
(4) More energetic response	N	0	1	0	0	0	0
(5) Attacks or bites probe	N	2	0	0	1	0	0
	MEDIAN SCORE	2.0	2.0	2.0	2.0	1.0**	1.5
PUPILLARY RESPONSE <sup>c</sup>	N	20	20	20	20	10**	20

GROUP		I			II		
DOSAGE (MG/KG/DAY) <sup>a</sup> - PHYSOSTIGMINE		0 (VEHICLE CONTROL)			0.75		
TEST		PRE-	POST-DOSAGE		PRE-	POST-DOSAGE	
		DOSAGE	15M	2HR	DOSAGE	15M	2HR
AIR RIGHTING REACTION							
(1) Lands with all feet on the ground	N	18	17	16	19	10	19
(2) Uncoordinated landing	N	2	3	4	1	5	0
(3) Lands on back	N	0	0	0	0	5	1
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.5#	1.0
VISUAL PLACING REACTION							
(1) Early extension of forelimbs	N	18	20	20	20	19	20
(2) Extension follows initial contact	N	2	0	0	0	1	0
(3) No extension after contact	N	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0
MALES							
	N	N	N	N	N	N	N
FORELIMB GRIP STRENGTH (MAXIMUM g)	MEAN	719	767	748	697	739	860
	± S.D.	± 100	± 150	± 175	± 123	± 195	± 102
HINDLIMB GRIP STRENGTH (MAXIMUM g)	MEAN	811	890	855	788	933	899
	± S.D.	± 128	± 88	± 87	± 168	± 88	± 133
FOOT SPREAD MEASURE (AVERAGE cm)	MEAN	7.8	9.4	8.5	8.3	11.0	10.2
	± S.D.	± 1.3	± 1.6	± 1.6	± 1.2	± 2.0	± 2.0*
BODY WEIGHT (g)	MEAN	359	372		360	368	
	± S.D.	± 83	± 81		± 56	± 48	
FEMALES							
	N	10	10	10	10	10	10
FORELIMB GRIP STRENGTH (MAXIMUM g)	MEAN	677	658	631	612	675	626
	± S.D.	± 223	± 117	± 149	± 196	± 198	± 168
HINDLIMB GRIP STRENGTH (MAXIMUM g)	MEAN	689	745	762	715	800	747
	± S.D.	± 152	± 130	± 154	± 122	± 128	± 113
FOOT SPREAD MEASURE (AVERAGE cm)	MEAN	7.7	10.2	9.0	6.9	9.2	7.4
	± S.D.	± 1.6	± 0.9	± 1.8	± 1.2	± 2.0	± 1.2*
BODY WEIGHT (g)	MEAN	235	235		235	237	
	± S.D.	± 47	± 48		± 46	± 44	

M = MINUTES      HR = HOURS

(n) = Score assigned to graded test items.

a. Dosage occurred once, on day 1 of the study.

b. Observation made at the home cage.

c. Number of rats showing a response.

d. Observation made in the open field.

\* Significantly different from the vehicle control group value ( $P \leq 0.05$ ). For items showing the combined data for both males and females, the differences were significant for both sexes.

\*\* Significantly different from the vehicle control group value ( $P \leq 0.01$ ). For items showing the combined data for both males and females, the differences were significant for both sexes.

# Significantly different from the vehicle control group value ( $P \leq 0.05$ ) for one, but not both sexes (for the items showing the combined data from both males and females).

## **Acrylamide (50 mg/kg)**

Statistically significant differences between dosage groups were observed for the following test items after eight dosages (one dosage every other day).

- Degree of Motility
- Gait
- Foot Splay
- Body Weight

GROUP		I				II			
DOSAGE (MG/KG/DAY) <sup>a</sup> - ACRYLAMIDE		0 (VEHICLE CONTROL)				50			
TEST		DOSAGE PERIOD				DOSAGE PERIOD			
		PRE-DOSAGE	DAY 1	DAY 9	DAY 17	PRE-DOSAGE	DAY 1	DAY 9	DAY 17
RATS TESTED	N	20	20	20	20	20	20	20	20
HOME CAGE BEHAVIOR									
Apparently sleeping	N	7	5	7	3	7	5	12	10
Awake, immobile, normal posture	N	8	6	7	10	11	10	6	10
Engaged in normal behavior	N	5	9	6	7	2	5	2	0#
Immobile, unusual posture	N	0	0	0	0	0	0	0	0
Engaged in unusual behavior	N	0	0	0	0	0	0	0	0
INVOLUNTARY BEHAVIOR <sup>b</sup>									
Not apparent	N	20	20	20	20	20	20	20	20
Stereotyped behavior pattern	N	0	0	0	0	0	0	0	0
Bizarre behavior	N	0	0	0	0	0	0	0	0
Tremors in limbs	N	0	0	0	0	0	0	0	0
Whole body tremors or spasms	N	0	0	0	0	0	0	0	0
Unusual posture	N	0	0	0	0	0	0	0	0
Tonic-clonic seizure	N	0	0	0	0	0	0	0	0
REACTION TO REMOVAL									
(1) Sits quietly	N	10	13	16	19	11	11	14	14
(2) Vocalization without resistance	N	3	5	3	0	5	6	5	5
(3) Runs around cage	N	7	2	1	1	1	3	1	1
(4) Freezes and rears, follows hand	N	0	0	0	0	3	0	0	0
(5) Tail and throat rattles	N	0	0	0	0	0	0	0	0
	MEDIAN SCORE	1.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0#
REACTION TO HANDLING									
(1) Quiet with no resistance	N	6	14	17	20	12	18	14	15
(2) Vocalization without resistance	N	5	3	1	0	3	0	2	2
(3) Tense and rigid	N	4	2	1	0	2	1	1	2
(4) Squirming and twisting	N	5	1	1	0	3	1	3	1
	MEDIAN SCORE	2.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
PALPEBRAL CLOSURE									
(1) Eyelids wide open	N	20	20	20	20	20	20	20	19
(2) Eyelids slightly drooping	N	0	0	0	0	0	0	0	1
(3) Eyelids drooping, half-closed	N	0	0	0	0	0	0	0	0
(4) Eyelids completely shut	N	0	0	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
PROMINENCE OF EYE									
Normal	N	20	20	20	20	20	20	20	20
Exophthalmos	N	0	0	0	0	0	0	0	0
Enophthalmos	N	0	0	0	0	0	0	0	0



GROUP		I				II			
DOSAGE (MG/KG/DAY) <sup>a</sup> - ACRYLAMIDE		0(VEHICLE CONTROL)				50			
TEST		DOSAGE PERIOD				DOSAGE PERIOD			
		PRE-DOSAGE	DAY 1	DAY 9	DAY 17	PRE-DOSAGE	DAY 1	DAY 9	DAY 17
LACRIMATION									
(1) No excessive lacrimation	N	20	20	20	20	20	20	20	20
(2) Slight	N	0	0	0	0	0	0	0	0
(3) Severe	N	0	0	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
SALIVATION									
(1) No excessive salivation	N	20	20	20	20	20	20	20	20
(2) Wet margin along submaxillary area	N	0	0	0	0	0	0	0	0
(3) Severe	N	0	0	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
PILOERECTION <sup>c</sup>	N	0	0	0	0	0	0	0	0
ABNORMAL RESPIRATION <sup>c</sup>	N	0	0	0	0	0	0	0	0
APPEARANCE									
(1) Clean and groomed	N	20	20	20	20	20	20	20	20
(2) Unkempt	N	0	0	0	0	0	0	0	0
(3) Stained by urine and feces	N	0	0	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
AROUSAL LEVEL									
(1) Very low (stuporous)	N	0	0	0	0	0	0	0	0
(2) Low (some exploratory movement)	N	0	0	0	1	0	0	0	0
(3) Apparently normal	N	20	20	20	19	20	20	20	20
(4) High (sudden darting and freezing)	N	0	0	0	0	0	0	0	0
(5) Very high (running and vocalization)	N	0	0	0	0	0	0	0	0
	MEDIAN SCORE	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
DEGREE OF MOTILITY									
(1) Apparently normal	N	20	20	20	20	20	20	20	0
(2) Moves with difficulty	N	0	0	0	0	0	0	0	20
(3) Unable to move	N	0	0	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0	1.0	2.0**
GAIT									
Normal	N	20	20	20	20	20	20	20	0**
Ataxic (swaying or lurching)	N	0	0	0	0	0	0	0	1
Exaggerated movements in limbs	N	0	0	0	0	0	0	0	19**
Tip-toe walk	N	0	0	0	0	0	0	0	0

GROUP		I				II			
DOSAGE (MG/KG/DAY) <sup>a</sup> - ACRYLAMIDE		0(VEHICLE CONTROL)				50			
TEST		DOSAGE PERIOD				DOSAGE PERIOD			
		PRE-DOSAGE	DAY 1	DAY 9	DAY 17	PRE-DOSAGE	DAY 1	DAY 9	DAY 17
INVOLUNTARY BEHAVIOR <sup>d</sup>									
None apparent	N	20	20	20	20	20	20	20	20
Stereotyped behavior pattern	N	0	0	0	0	0	0	0	0
Bizarre behavior	N	0	0	0	0	0	0	0	0
Tremors in limbs	N	0	0	0	0	0	0	0	0
Whole body tremors or spasms	N	0	0	0	0	0	0	0	0
Unusual posture	N	0	0	0	0	0	0	0	0
Tonic-clonic seizure	N	0	0	0	0	0	0	0	0
FECAL BOLUSES	MEAN	0.7	1.2	1.0	1.3	0.5	0.8	1.0	1.2
	± S.D.	± 0.9	± 1.2	± 1.5	± 2.0	± 0.9	± 1.3	± 1.3	± 1.9
URINE POOLS	MEAN	0.6	1.0	0.8	0.9	0.5	0.6	0.9	1.2
	± S.D.	± 0.9	± 1.1	± 0.8	± 0.9	± 0.9	± 0.8	± 1.1	± 1.1
REACTION TO VISUAL STIMULUS									
(1) No reaction	N	6	6	6	8	5	8	8	4
(2) Moves toward or away from stimulus	N	14	14	14	12	15	12	12	10
(3) Startle response or freezing	N	0	0	0	0	0	0	0	6
(4) More energetic response	N	0	0	0	0	0	0	0	0
(5) Attacks or bites probe	N	0	0	0	0	0	0	0	0
	MEDIAN SCORE	2.0	2.50	2.0	2.0	2.0	2.0	2.0	2.0
REACTION TO TACTILE STIMULUS									
(1) No reaction	N	16	12	10	13	14	12	13	10
(2) Moves towards or away from stimulus	N	4	8	6	3	5	8	7	5
(3) Startle response or freezing	N	0	0	4	4	1	0	0	5
(4) More enrtgetic response	N	0	0	0	0	0	0	0	0
(5) Attacks or bites probe	N	0	0	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.5	1.0	1.0	1.0	1.0	1.0
REACTION TO AUDITORY STIMULUS									
(1) No reaction	N	0	0	0	1	2	2	0	2
(2) Moves towards or away from stimulus	N	1	2	2	0	1	0	1	1
(3) Startle response or freezing	N	19	18	17	19	15	18	19	16
(4) More energetic response	N	0	0	1	0	2	0	0	1
(5) Jumps at or away from the noise	N	0	0	0	0	0	0	0	0
	MEDIAN SCORE	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
REACTION TO TAIL PINCH									
(1) No reaction	N	4	9	4	6	5	5	2	3
(2) Moves toward or away from stimulus	N	9	7	10	7	11	12	7	7
(3) Startle response or freezing	N	3	4	4	7	3	3	10	7
(4) More energetic response	N	4	0	2	0	1	0	1	3
(5) Attacks or bites probe	N	0	0	0	0	0	0	0	0
	MEDIAN SCORE	2.0	2.0	2.0	2.0	2.0	2.0	3.0	2.5#
PUPILLARY RESPONSE <sup>c</sup>	N	20	20	20	20	20	20	20	20

GROUP		I				II			
DOSAGE (MG/KG/DAY) <sup>a</sup> - ACRYLAMIDE		0 (VEHICLE CONTROL)				50			
TEST		DOSAGE PERIOD				DOSAGE PERIOD			
		PRE-DOSAGE	DAY 1	DAY 9	DAY 17	PRE-DOSAGE	DAY 1	DAY 9	DAY 17
AIR RIGHTING REACTION									
(1) Lands with all feed on the ground	N	20	16	16	18	20	19	20	17
(2) Uncoordinated landing	N	0	4	4	2	0	1	0	2
(3) Lands on back	N	0	0	0	0	0	0	0	1
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
VISUAL PLACING REACTION									
(1) Early extension of forelimbs	N	19	20	20	20	18	20	20	20
(2) Extension follows initial contact	N	1	0	0	0	2	0	0	0
(3) No extension after contact	N	0	0	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
MALES									
	N	10	10	10	10	10	10	10	10
FORELIMB GRIP STRENGTH (MAXIMUM g)	MEAN	631	688	821	792	632	704	715	805
	± S.D.	± 115	± 184	± 123	± 191	± 134	± 202	± 240	± 216
HINDLIMB GRIP STRENGTH (MAXIMUM g)	MEAN	800	882	916	936	763	914	886	882
	± S.D.	± 107	± 112	± 117	± 111	± 129	± 112	± 127	± 129
HINDLIMB FOOT SPPLAY (AVERAGE cm)	MEAN	7.6	8.9	8.7	7.8	7.3	8.3	9.6	12.3
	± S.D.	± 1.7	± 2.6	± 2.3	± 1.9	± 2.0	± 2.5	± 1.8	± 1.7**
BODY WEIGHT (g)	MEAN	321	348	376	397	314	342	347	345
	± S.D.	± 55	± 54	± 52	± 51	± 52	± 51	± 41	± 35**
FEMALES									
	N	10	10	10	10	10	10	10	10
FORELIMB GRIP STRENGTH (MAXIMUM g)	MEAN	554	604	644	533	605	558	632	527
	± S.D.	± 184	± 174	± 196	± 118	± 226	± 204	± 159	± 172
HINDLIMB GRIP STRENGTH (MAXIMUM g)	MEAN	658	736	740	756	648	766	680	702
	± S.D.	± 99	± 92	± 192	± 164	± 160	± 106	± 130	± 168
FOOT SPPLAY MEASURE (AVERAGE cm)	MEAN	7.0	8.2	7.2	7.4	7.0	7.5	9.6	11.7
	± S.D.	± 0.9	± 1.4	± 0.8	± 1.2	± 0.9	± 1.2	± 1.4**	± 1.1**
BODY WEIGHT (g)	MEAN	220	230	236	240	223	235	232	228
	± S.D.	± 50	± 47	± 43	± 43	± 50	± 45	± 34	± 30

DAY = DAY OF THE STUDY

(n) = Score assigned to graded test items.

a. Dosage occurred once every other day of the study, beginning on day 1 and ending on day 15 (total of eight dosages).

b. Observation made at the home cage.

c. Number of rats showing a response.

d. Observation made in the open field.

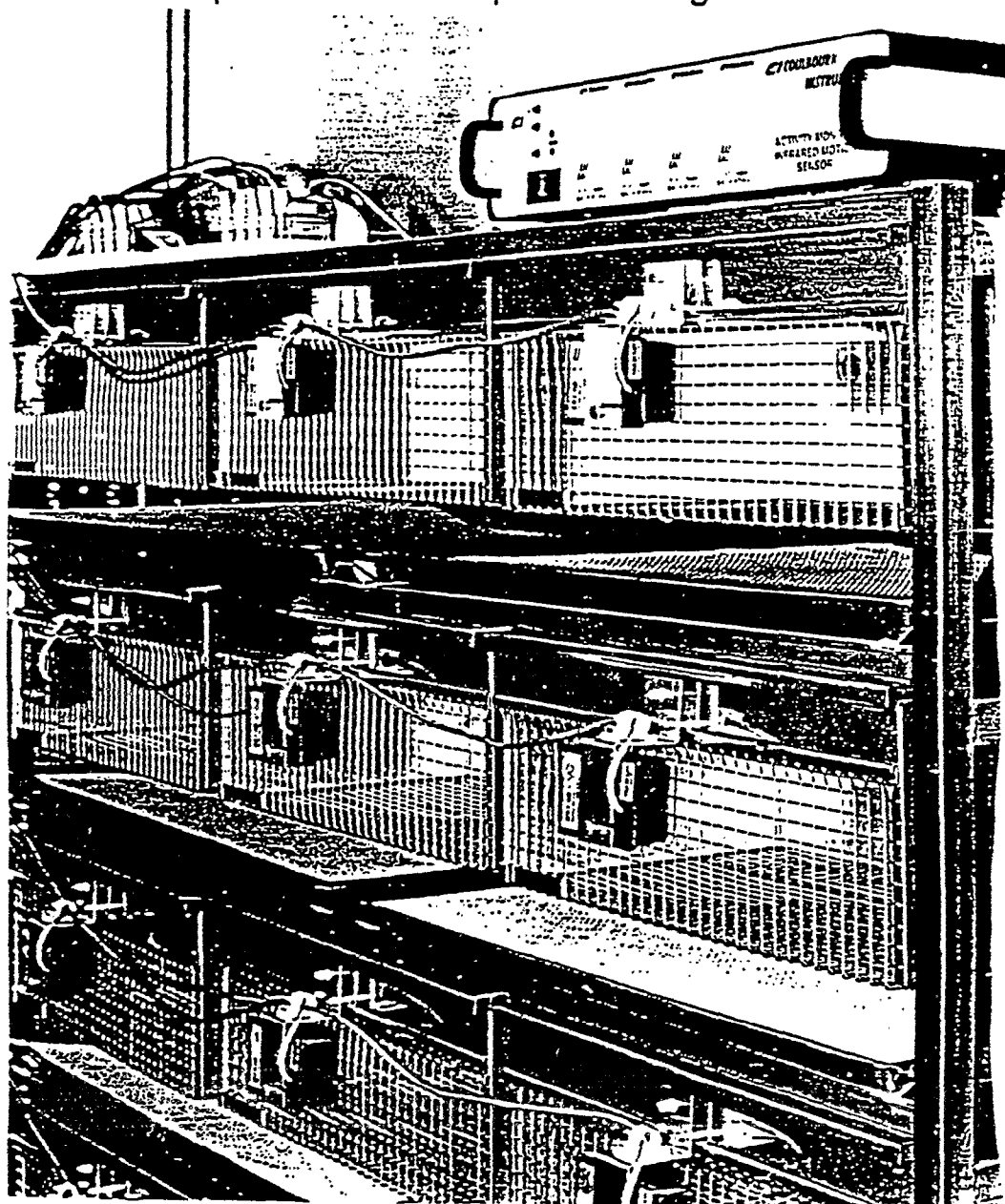
\* Significantly different from the vehicle control group value ( $P \leq 0.05$ ). For items showing the combined data for both males and females, the differences were significant for both sexes.

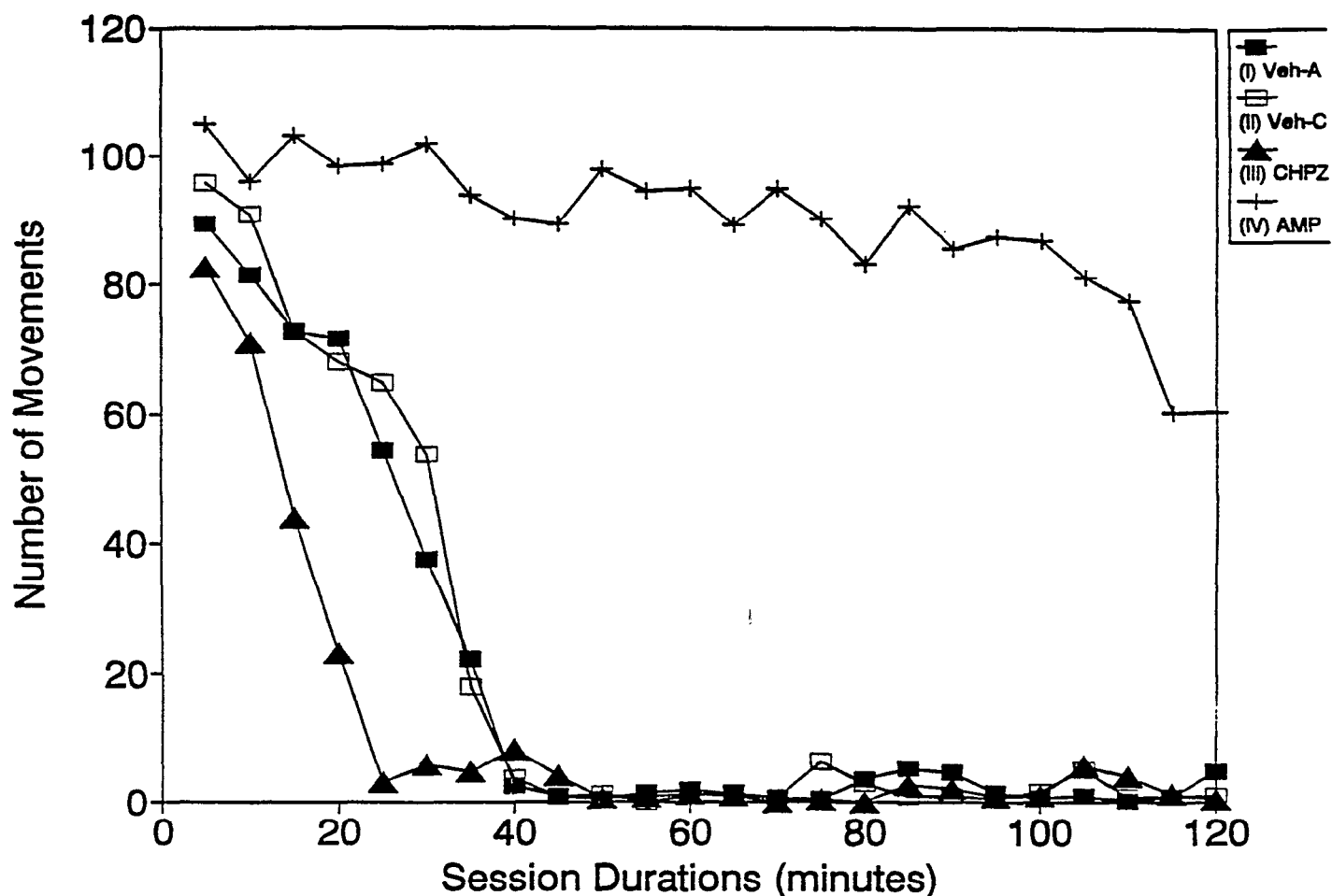
\*\* Significantly different from the vehicle control group value ( $P \leq 0.01$ ). For items showing the combined data for both males and females, the differences were significant for both sexes.

# Significantly different from the vehicle control group value ( $P \leq 0.05$ ) for one, but not both sexes (for the items showing the combined data from both males and females).

## MOTOR ACTIVITY

Standard rat cages (double width) were used for the motor activity tests. The response of the passive infrared sensor was proportional to the extent of movement and was based on changes in the pattern of heat in the cage. Sets of eight sensors were connected to a distribution box from which a single cable led to the unit containing analog-to-digital (A/D) converters and a power supply; the unit could monitor 32 sensors. The A/D converters sampled the output of the sensors every 200 milliseconds under the control of a microcomputer. Using predefined thresholds, the computer classified the samples as representing no movement, a small movement, or a large movement. Two or more consecutive samples in the same movement category were defined as a discrete movement and the number of movements were summed over five minute blocks along with the total time spent in each category. A calibration device and associated software were used to standardize the output of the sensors prior to testing.





The data for the large and small movement categories were combined and then analyzed using a repeated measures analysis of variance with the probabilities corrected by the Greenhouse-Geiser procedure. The groups administered the test substances, amphetamine (AMP) and chlorpromazine (CHPZ), showed statistically significant differences from their respective controls in total number of movements (shown here) and time spent in movement as well as in the change throughout the session.

**APPENDIX 5**  
**MOTOR ACTIVITY POSITIVE CONTROL #1**

**Validation of a Functional Observational Battery  
and Motor Activity Measure  
Using Positive Test Substances**

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## ABSTRACT

The EPA guidelines for neurotoxicity assessments are expected to be finalized by January 1991. These assessments are expected to include a Functional Observational Battery (FOB) and motor activity measurement. To validate the FOB a single dosage of p,p' DDT (75 mg/kg, p.o.) and physostigmine monosalicylate (0.75 mg/kg, IP), and repeated dosages of acrylamide (50 mg/kg, IP, every other day) were used. The test substances and their vehicles were administered to separate groups of adult male (10) and female (10) rats (CrI:CD@BR VAF/Plus®). Each test substance produced different patterns of effects. The rats administered DDT did not differ from controls 1 hour after administration; after 5 hours, tremors were evident throughout the body, reactivity to a visual stimulus was increased, and grip strength and foot splay were decreased. Physostigmine produced increased salivation, miosis, tremors in the limbs and changes in gait at 15 minutes after administration but not at 2 hours. Acrylamide produced an increase in foot splay and changes in gait following eight dosages. To validate the motor activity assessment, groups of seven male and female rats were administered chlorpromazine (2.0 mg/kg, IP) and its vehicle 70 minutes prior to testing, or amphetamine (1.0 mg/kg, IP) and its vehicle immediately before the test session. Motor activity was assessed for a 2-hour period. All groups diminished their activity throughout the session, but the habituation was less evident in the group given amphetamine. In contrast, the groups given chlorpromazine decreased their activity at a faster rate than the two control groups.



## FUNCTIONAL OBSERVATIONAL BATTERY

The test items were principally derived from published protocols (Haggerty, G.C., J. Am. Coll. Toxicol. 1989, 8, 53-69; Moser, V. C., J. Am. Coll. Toxicol. 1989, 8, 85-93; Moser, V. C. et al., Fundam. Appl. Toxicol., 1988, 11, 189-206; O'Donoghue, J. L., J. Am. Coll. Toxicol., 1989, 8, 97-115) and were based on the current EPA guideline (40 CFR 798.6050) and proposed revisions. The grip-strength device was patterned after the device described by Mattson et al. (Fundam. Appl. Toxicol., 1986, 6, 175-181), a modification of the apparatus described by Meyer et al. (Neurobehav. Toxicol., 1979, 1, 233-236). The tests were conducted under GLP guidelines. Statistical analyses for dosage effects were performed separately for each item at each test session and for each sex. Items with descriptive or binary elements were first analyzed as  $2 \times n$  contingency tables, and significant overall results were followed by  $2 \times 2$  comparisons for each element. Items with graded scores and count data were evaluated using the Kruskal-Wallis procedure. For the tables presented here, the data for both sexes were tabulated together for the items with those data types. Items with interval data were analyzed by ANOVA or the Kruskal-Wallis procedure depending upon the outcome of Bartlett's test for equality of variance. The data for males and females are displayed separately for those items.

## **DDT (75 mg/kg)**

Statistically significant differences between dosage groups were observed for the following test items five hours after dosage.

- Home Cage Behavior
- Involuntary Behavior at the Home Cage and in the Open Field
- Reaction to a Visual Stimulus
- Grip Strength
- Foot Splay

GROUP		I			II		
DOSAGE (MG/KG/DAY) <sup>a</sup> - DDT		0 (VEHICLE CONTROL)			75		
TEST		PRE-DOSAGE	POST-DOSAGE		PRE-DOSAGE	POST-DOSAGE	
			1HR	5HR		1HR	5HR
RATS TESTED	N	20	20	20	20	20	20
HOME CAGE BEHAVIOR							
Apparently sleeping	N	5	4	6	6	1	1
Awake, immobile, normal posture	N	8	9	12	8	10	1**
Engaged in normal behavior	N	7	7	2	6	9	0
Immobile, unusual behavior	N	0	0	0	0	0	0
Engaged in unusual behavior	N	0	0	0	0	0	18**
INVOLUNTARY BEHAVIOR <sup>b</sup>							
Not apparent	N	20	20	20	20	20	2**
Stereotyped behavior pattern	N	0	0	0	0	0	1
Bizarre behavior	N	0	0	0	0	0	0
Tremors in limbs	N	0	0	0	0	0	0
Whole body tremors or spasms	N	0	0	0	0	0	17**
Unusual posture	N	0	0	0	0	0	0
Tonic-clonic seizure	N	0	0	0	0	0	0
REACTION TO REMOVAL							
(1) Sits quietly	N	10	12	11	11	10	14
(2) Vocalization without resistance	N	8	6	7	4	6	2
(3) Runs around cage	N	2	2	2	4	4	3
(4) Freezes and rears, follows hand	N	0	0	0	1	0	0
(5) Tail and throat rattles	N	0	0	0	0	0	1
MEDIAN SCORE		1.5	1.0	1.0	1.0	1.5	1.0
REACTION TO HANDLING							
(1) Quiet with no resistance	N	14	16	16	11	15	15
(2) Vocalization without resistance	N	2	3	3	3	1	1
(3) Tense and rigid	N	3	0	1	3	1	0
(4) Squirming and twisting	N	1	1	0	3	3	4
MEDIAN SCORE		1.0	1.0	1.0	1.0	1.0	1.0
PALPEBRAL CLOSURE							
(1) Eyelids wide open	N	20	20	20	20	20	20
(2) Eyelids slightly drooping	N	0	0	0	0	0	0
(3) Eyelids drooping, half-closed	N	0	0	0	0	0	0
(4) Eyelids completely shut	N	0	0	0	0	0	0
MEDIAN SCORE		1.0	1.0	1.0	1.0	1.0	1.0
PROMINENCE OF EYE							
Normal	N	20	20	20	20	20	20
Exophthalmos	N	0	0	0	0	0	0
Enophthalmos	N	0	0	0	0	0	0

GROUP		I			II		
DOSAGE (MG/KG/DAY) <sup>a</sup> - DDT		0(VEHICLE CONTROL)			75		
TEST		PRE- DOSAGE	POST-DOSAGE 1HR	5HR	PRE- DOSAGE	POST-DOSAGE 1HR	5HR
LACRIMATION							
(1) No excessive lacrimation	N	20	20	20	20	20	20
(2) Slight	N	0	0	0	0	0	0
(3) Severe	N	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0
SALIVATION							
(1) No excessive salivation	N	20	19	20	20	20	20
(2) Wet margin along submaxillary area	N	0	0	0	0	0	0
(3) Severe	N	0	1	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0
PILOERECTION <sup>c</sup>	N	0	0	0	0	0	0
ABNORMAL RESPIRATION <sup>c</sup>	N	0	0	0	0	0	0
APPEARANCE							
(1) Clean and groomed	N	20	20	20	20	20	20
(2) Unkempt	N	0	0	0	0	0	0
(3) Stained by urine and feces	N	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0
AROUSAL LEVEL							
(1) Very low (stuporous)	N	0	0	0	0	0	0
(2) Low (some exploratory movement)	N	0	0	3	0	0	0
(3) Apparently normal	N	20	20	17	20	20	18
(4) High (sudden darting and freezing)	N	0	0	0	0	0	2
(5) Very high (running, vocalization)	N	0	0	0	0	0	0
	MEDIAN SCORE	3.0	3.0	3.0	3.0	3.0	3.0
DEGREE OF MOTILITY							
(1) Apparently normal	N	20	20	20	20	20	11
(2) Moves with difficulty	N	0	0	0	0	0	9
(3) Unable to move	N	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0
GAIT							
Normal	N	20	20	20	20	20	13
Ataxic (swaying or lurching)	N	0	0	0	0	0	0
Exaggerated movements in limbs	N	0	0	0	0	0	7
Tip-toe walk	N	0	0	0	0	0	0

GROUP		I			II		
DOSAGE (MG/KG/DAY) <sup>a</sup> - DDT		0(VEHICLE CONTROL)			75		
TEST		PRE-	POST-DOSAGE		PRE-	POST-DOSAGE	
		DOSAGE	1HR	5HR	DOSAGE	1HR	5HR
INVOLUNTARY BEHAVIOR <sup>d</sup>							
None apparent	N	20	20	20	20	20	2**
Stereotyped behavior pattern	N	0	0	0	0	0	0
Bizarre behavior	N	0	0	0	0	0	0
Tremor in limbs	N	0	0	0	0	0	1
Whole body tremors or spasms	N	0	0	0	0	0	17**
Unusual posture	N	0	0	0	0	0	0
Tonic-clonic seizure	N	0	0	0	0	0	0
FECAL BOLUSES	MEAN	0.4	0.7	0.6	1.6	0.9	1.7
	± S.D.	± 0.8	± 1.3	± 1.2	± 2.3	± 1.3	± 1.9#
URINE POOLS	MEAN	1.0	1.0	0.5	1.0	0.9	1.0
	± S.D.	± 0.9	± 0.9	± 0.5	± 0.8	± 1.0	± 1.0
REACTION TO VISUAL STIMULUS							
(1) No reaction	N	3	5	7	5	2	3
(2) Moves toward or away from stimulus	N	17	15	12	13	18	6
(3) Startle response or freezing	N	0	0	1	2	0	10
(4) More energetic response	N	0	0	0	0	0	1
(5) Attacks or bites probe	N	0	0	0	0	0	0
	MEDIAN SCORE	2.0	2.0	2.0	2.0	2.0	3.0*
REACTION TO TACTILE STIMULUS							
(1) No reaction	N	9	7	13	10	13	11
(2) Moves toward or away from stimulus	N	4	3	2	7	4	5
(3) Startle response or freezing	N	7	10	5	3	3	4
(4) More energetic response	N	0	0	0	0	0	0
(5) Attacks or bites probe	N	0	0	0	0	0	0
	MEDIAN SCORE	1.5	2.5	1.0	1.5	1.0#	1.0
REACTION TO AUDITORY STIMULUS							
(1) No reaction	N	1	2	0	0	1	0
(2) Moves toward or away from response	N	0	1	0	2	1	0
(3) Startle response or freezing	N	18	15	19	18	18	16
(4) More energetic response	N	1	2	1	0	0	4
(5) Jumps at or away from the noise	N	0	0	0	0	0	0
	MEDIAN SCORE	3.0	3.0	3.0	3.0	3.0	3.0
REACTION TO TAIL PINCH							
(1) No reaction	N	11	11	14	6	7	9
(2) Moves toward or away from stimulus	N	5	7	5	8	8	10
(3) Startle response or freezing	N	3	2	1	6	5	1
(4) More energetic response	N	1	0	0	0	0	0
(5) Attacks or bites probe	N	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	2.0	2.0	2.0
PUPILLARY RESPONSE <sup>c</sup>	N	19	19	20	20	20	20

GROUP DOSAGE (MG/KG/DAY) <sup>a</sup> - DDT		I 0(VEHICLE CONTROL)			II 75		
TEST		PRE- DOSAGE	POST-DOSAGE 1HR	5HR	PRE- DOSAGE	POST-DOSAGE 1HR	5HR
AIR RIGHTING REACTION							
(1) Lands with all feet on the ground	N	19	20	19	20	20	16
(2) Uncoordinated landing	N	1	0	1	0	0	4
(3) Lands on back	N	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0
VISUAL PLACING REACTION							
(1) Early extension of forelimbs	N	20	20	20	20	20	20
(2) Extension follows initial contact	N	0	0	0	0	0	0
(3) No extension after contact	N	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0
MALES:		N	10	10	10	10	10
FORELIMB GRIP STRENGTH (MAXIMUM g)	MEAN	911	908	782	900	880	505
	± S.D.	± 156	± 114	± 113	± 113	± 155	± 219**
HINDLIMB GRIP STRENGTH (MAXIMUM g)	MEAN	913	938	884	844	944	804
	± S.D.	± 92	± 88	± 163	± 150	± 104	± 164
FOOT SPREAD MEASURE (AVERAGE cm)	MEAN	10.1	8.7	8.9	9.1	8.1	6.6
	± S.D.	± 3.2	± 2.3	± 1.4	± 2.3	± 2.1	± 0.6**
BODY WEIGHT (g)	MEAN	522	558		551	558	
	± S.D.	± 77	± 74		± 78	± 77	
FEMALES:		N	10	10	10	10	10
FORELIMB GRIP STRENGTH (MAXIMUM g)	MEAN	793	732	646	823	792	590
	± S.D.	± 163	± 180	± 155	± 154	± 213	± 151
HINDLIMB GRIP STRENGTH (MAXIMUM g)	MEAN	634	689	768	687	659	627
	± S.D.	± 153	± 169	± 108	± 94	± 188	± 162*
FOOT SPREAD MEASURE (AVERAGE cm)	MEAN	7.4	7.5	7.3	7.7	6.7	5.7
	± S.D.	± 2.2	± 1.6	± 1.4	± 1.7	± 1.6	± 1.2*
BODY WEIGHT (g)	MEAN	276	279		278	289	
	± S.D.	± 23	± 20		± 25	± 20	

HR = HOURS

(n) = Score assigned to graded test items.

- Dosage occurred once, on day 1 of the study.
- Observation made at the home cage.
- Number of rats showing a response.
- Observation made in the open field.

\* Significantly different from the vehicle control group value ( $P \leq 0.05$ ). For the items showing the combined data from both males and females, the differences were significant for both sexes.

\*\* Significantly different from the vehicle control group value ( $P \leq 0.01$ ). For the items showing the combined data from both males and females, the differences were significant for both sexes.

# Significantly different from the vehicle control group value ( $P \leq 0.05$ ) for one, but not both sexes (for the items showing the combined data from both males and females)

### **Physostigmine (0.75 mg/kg)**

Statistically significant differences between dosage groups were observed for the following test items 15 minutes after dosage.

- Home Cage Behavior
- Involuntary Behavior at the Home Cage and in the Open Field
- Reaction to Removal
- Salivation
- Arousal Level
- Degree of Motility
- Gait
- Reaction to Tail Pinch
- Pupillary Response

GROUP		I			II		
DOSAGE (MG/KG/DAY) <sup>a</sup> - PHYSOSTIGMINE		0 (VEHICLE CONTROL)			0.75		
TEST		PRE-	POST-DOSAGE		PRE-	POST-DOSAGE	
		DOSAGE	15M	2HR	DOSAGE	15M	2HR
RATS TESTED	N	20	20	20	20	20	20
HOME CAGE BEHAVIOR							
Apparently sleeping	N	6	0	11	3	0	3
Awake, immobile, normal posture	N	9	12	6	12	6	13
Engaged in normal behavior	N	5	8	3	5	0	4
Immobile, unusual behavior	N	0	0	0	0	0	0
Engaged in unusual behavior	N	0	0	0	0	14**	0
INVOLUNTARY BEHAVIOR <sup>b</sup>							
Not apparent	N	20	20	20	20	4**	20
Stereotyped behavior pattern	N	0	0	0	0	0	0
Bizarre behavior	N	0	0	0	0	0	0
Tremors in limbs	N	0	0	0	0	16**	0
Whole body tremors or spasms	N	0	0	0	0	0	0
Unusual posture	N	0	0	0	0	0	0
Tonic-clonic seizure	N	0	0	0	0	0	0
REACTION TO REMOVAL							
(1) Site quietly	N	13	10	16	12	20	13
(2) Vocalization without resistance	N	2	5	2	4	0	6
(3) Runs around cage	N	5	4	2	3	0	1
(4) Freezes and rears, follows hand	N	0	1	0	1	0	0
(5) Tail and throat rattles	N	0	0	0	0	0	0
MEDIAN SCORE		1.0	1.5	1.0	1.0	1.0*	1.0
REACTION TO HANDLING							
(1) Quiet with no resistance	N	11	15	19	11	19	18
(2) Vocalization without resistance	N	2	0	0	2	0	1
(3) Tense and rigid	N	2	1	1	1	0	0
(4) Squirming and twisting	N	5	4	0	6	1	1
MEDIAN SCORE		1.0	1.0	1.0	1.0	1.0	1.0
PALPEBRAL CLOSURE							
(1) Eyelids wide open	N	20	20	20	20	20	20
(2) Eyelids slightly drooping	N	0	0	0	0	0	0
(3) Eyelids drooping, half-closed	N	0	0	0	0	0	0
(4) Eyelids completely shut	N	0	0	0	0	0	0
MEDIAN SCORE		1.0	1.0	1.0	1.0	1.0	1.0
PROMINENCE OF EYE							
Normal	N	20	20	20	20	20	20
Exophthalmos	N	0	0	0	0	0	0
Enophthalmos	N	0	0	0	0	0	0



GROUP		I			II		
DOSAGE (MG/KG/DAY) <sup>a</sup> - PHYSOSTIGMINE		0 (VEHICLE CONTROL)			0.75		
TEST		PRE-DOSAGE	POST-DOSAGE 15M	2HR	PRE-DOSAGE	POST-DOSAGE 15M	2HR
<b>LACRIMATION</b>							
(1) No excessive lacrimation	N	20	20	20	20	20	20
(2) Slight	N	0	0	0	0	0	0
(3) Severe	N	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0
<b>SALIVATION</b>							
(1) No excessive salivation	N	20	20	19	20	11	19
(2) Wet margin along submaxillary area	N	0	0	1	0	3	1
(3) Severe	N	0	0	0	0	6	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0 <sup>a</sup>	1.0
<b>PILOERECTION<sup>c</sup></b>							
	N	0	0	0	0	0	0
<b>ABNORMAL RESPIRATION<sup>c</sup></b>							
	N	0	0	0	0	0	0
<b>APPEARANCE</b>							
(1) Clean and groomed	N	20	20	20	20	20	20
(2) Unkempt	N	0	0	0	0	0	0
(3) Stained by urine and feces	N	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0
<b>AROUSAL LEVEL</b>							
(1) Very low (stuporous)	N	0	0	0	0	0	0
(2) Low (some exploratory movement)	N	0	0	0	0	6	0
(3) Apparently normal	N	20	20	20	20	14	20
(4) High (sudden darting and freezing)	N	0	0	0	0	0	0
(5) Very high (running, vocalization)	N	0	0	0	0	0	0
	MEDIAN SCORE	3.0	3.0	3.0	3.0	3.0 <sup>a</sup>	3.0
<b>DEGREE OF MOTILITY</b>							
(1) Apparently normal	N	20	20	20	20	4	20
(2) Moves with difficulty	N	0	0	0	0	16	0
(3) Unable to move	N	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	2.0 <sup>a*</sup>	1.0
<b>GAIT</b>							
Normal	N	20	20	20	19	3 <sup>**</sup>	20
Ataxic (awaying or lurching)	N	0	0	0	0	0	0
Exaggerated movements in limbs	N	0	0	0	0	17 <sup>**</sup>	0
Tip-toe walk	N	0	0	0	1	0	0

GROUP		I			II		
DOSAGE (MG/KG/DAY) <sup>a</sup> - PHYSOSTIGMINE		0 (VEHICLE CONTROL)			0.75		
TEST		PRE-	POST-DOSAGE		PRE-	POST-DOSAGE	
		DOSAGE	15M	2HR	DOSAGE	15M	2HR
INVOLUNTARY BEHAVIOR <sup>d</sup>							
None apparent	N	20	20	20	20	2**	20
Stereotyped behavior pattern	N	0	0	0	0	0	0
Bizarre behavior	N	0	0	0	0	0	0
Tremors in limbs	N	0	0	0	0	18**	0
Whole body tremors or spasms	N	0	0	0	0	0	0
Unusual posture	N	0	0	0	0	0	0
Tonic-clonic seizure	N	0	0	0	0	0	0
FECAL BOLUSES	MEAN	0.5	0.7	1.2	1.1	1.0	0.8
	± S.D.	± 0.9	± 1.4	± 1.3	± 1.0#	± 0.9	± 0.9
URINE POOLS	MEAN	1.0	0.9	0.7	0.6	1.0	0.6
	± S.D.	± 0.9	± 1.0	± 0.8	± 0.9	± 1.0	± 0.7
REACTION TO VISUAL STIMULUS							
(1) No reaction	N	6	8	8	2	7	8
(2) Moves towards or away from stimulus	N	14	10	12	18	13	9
(3) Startle response or freezing	N	0	2	0	0	0	3
(4) More energetic response	N	0	0	0	0	0	0
(5) Attacks or bites probe	N	0	0	0	0	0	0
	MEDIAN SCORE	2.0	2.0	2.0	2.0	2.0	2.0
REACTION TO TACTILE STIMULUS							
(1) No reaction	N	16	12	17	13	18	15
(2) Moves towards or away from stimulus	N	4	6	2	6	0	4
(3) Startle response or freezing	N	0	2	1	1	2	1
(4) More energetic response	N	0	0	0	0	0	0
(5) Attacks or bites probe	N	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0
REACTION TO AUDITORY STIMULUS							
(1) No reaction	N	1	1	2	0	3	1
(2) Moves toward or away from stimulus	N	2	0	1	1	0	1
(3) Startle response or freezing	N	16	19	17	19	12	18
(4) More energetic response	N	0	0	0	0	5	0
(5) Jumps at or away from the noise	N	0	0	0	0	0	0
	MEDIAN SCORE	3.0	3.0	3.0	3.0	3.0	3.0
REACTION TO TAIL PINCH							
(1) No reaction	N	8	6	9	8	18	10
(2) Moves towards or away from stimulus	N	6	9	10	6	0	5
(3) Startle response or freezing	N	4	4	1	5	2	5
(4) More energetic response	N	0	1	0	0	0	0
(5) Attacks or bites probe	N	2	0	0	1	0	0
	MEDIAN SCORE	2.0	2.0	2.0	2.0	1.0**	1.5
PUPILLARY RESPONSE <sup>c</sup>	N	20	20	20	20	10**	20

GROUP		I			II		
DOSAGE (MG/KG/DAY) <sup>a</sup> - PHYSOSTIGMINE		0 (VEHICLE CONTROL)			0.75		
TEST		PRE-	POST-DOSAGE		PRE-	POST-DOSAGE	
		DOSAGE	15M	2HR	DOSAGE	15M	2HR
AIR RIGHTING REACTION							
(1) Lands with all feet on the ground	N	18	17	16	19	10	19
(2) Uncoordinated landing	N	2	3	4	1	5	0
(3) Lands on back	N	0	0	0	0	5	1
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.5#	1.0
VISUAL PLACING REACTION							
(1) Early extension of forelimbs	N	18	20	20	20	19	20
(2) Extension follows initial contact	N	2	0	0	0	1	0
(3) No extension after contact	N	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0
MALES							
	N	N	N	N	N	N	N
FORELIMB GRIP STRENGTH (MAXIMUM g)	MEAN	719	767	748	697	739	860
	± S.D.	± 100	± 150	± 175	± 123	± 195	± 102
HINDLIMB GRIP STRENGTH (MAXIMUM g)	MEAN	811	890	855	788	933	899
	± S.D.	± 128	± 88	± 87	± 168	± 88	± 133
FOOT SPREAD MEASURE (AVERAGE cm)	MEAN	7.8	9.4	8.5	8.3	11.0	10.2
	± S.D.	± 1.3	± 1.6	± 1.6	± 1.2	± 2.0	± 2.0*
BODY WEIGHT (g)	MEAN	359	372		360	368	
	± S.D.	± 83	± 81		± 56	± 48	
FEMALES							
	N	10	10	10	10	10	10
FORELIMB GRIP STRENGTH (MAXIMUM g)	MEAN	677	658	631	612	675	626
	± S.D.	± 223	± 117	± 149	± 196	± 198	± 168
HINDLIMB GRIP STRENGTH (MAXIMUM g)	MEAN	689	745	762	715	800	747
	± S.D.	± 152	± 130	± 154	± 122	± 128	± 113
FOOT SPREAD MEASURE (AVERAGE cm)	MEAN	7.7	10.2	9.0	6.9	9.2	7.4
	± S.D.	± 1.6	± 0.9	± 1.8	± 1.2	± 2.0	± 1.2*
BODY WEIGHT (g)	MEAN	235	235		235	237	
	± S.D.	± 47	± 48		± 46	± 44	

M = MINUTES      HR = HOURS

(n) = Score assigned to graded test items.

- Dosage occurred once, on day 1 of the study.
- Observation made at the home cage.
- Number of rats showing a response.
- Observation made in the open field.

\* Significantly different from the vehicle control group value ( $P < 0.05$ ). For items showing the combined data for both males and females, the differences were significant for both sexes.

\*\* Significantly different from the vehicle control group value ( $P < 0.01$ ). For items showing the combined data for both males and females, the differences were significant for both sexes.

# Significantly different from the vehicle control group value ( $P < 0.05$ ) for one, but not both sexes (for the items showing the combined data from both males and females).

## **Acrylamide (50 mg/kg)**

Statistically significant differences between dosage groups were observed for the following test items after eight dosages (one dosage every other day).

- Degree of Motility
- Gait
- Foot Splay
- Body Weight

GROUP		I				II			
DOSAGE (MG/KG/DAY) <sup>a</sup> - ACRYLAMIDE		0 (VEHICLE CONTROL)				50			
TEST		DOSAGE PERIOD				DOSAGE PERIOD			
		PRE-DOSAGE	DAY 1	DAY 9	DAY 17	PRE-DOSAGE	DAY 1	DAY 9	DAY 17
RATS TESTED	N	20	20	20	20	20	20	20	20
HOME CAGE BEHAVIOR									
Apparently sleeping	N	7	5	7	3	7	5	12	10
Awake, immobile, normal posture	N	8	6	7	10	11	10	6	10
Engaged in normal behavior	N	5	9	6	7	2	5	2	0#
Immobile, unusual posture	N	0	0	0	0	0	0	0	0
Engaged in unusual behavior	N	0	0	0	0	0	0	0	0
INVOLUNTARY BEHAVIOR <sup>b</sup>									
Not apparent	N	20	20	20	20	20	20	20	20
Stereotyped behavior pattern	N	0	0	0	0	0	0	0	0
Bizarre behavior	N	0	0	0	0	0	0	0	0
Tremors in limbs	N	0	0	0	0	0	0	0	0
Whole body tremors or spasms	N	0	0	0	0	0	0	0	0
Unusual posture	N	0	0	0	0	0	0	0	0
Tonic-clonic seizure	N	0	0	0	0	0	0	0	0
REACTION TO REMOVAL									
(1) Sits quietly	N	10	13	16	19	11	11	14	14
(2) Vocalization without resistance	N	3	5	3	0	5	6	5	5
(3) Runs around cage	N	7	2	1	1	1	3	1	1
(4) Freezes and rears, follows hand	N	0	0	0	0	3	0	0	0
(5) Tail and throat rattles	N	0	0	0	0	0	0	0	0
	MEDIAN SCORE	1.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0#
REACTION TO HANDLING									
(1) Quiet with no resistance	N	6	14	17	20	12	18	14	15
(2) Vocalization without resistance	N	5	3	1	0	3	0	2	2
(3) Tense and rigid	N	4	2	1	0	2	1	1	2
(4) Squirming and twisting	N	5	1	1	0	3	1	3	1
	MEDIAN SCORE	2.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
PALPEBRAL CLOSURE									
(1) Eyelids wide open	N	20	20	20	20	20	20	20	19
(2) Eyelids slightly drooping	N	0	0	0	0	0	0	0	1
(3) Eyelids drooping, half-closed	N	0	0	0	0	0	0	0	0
(4) Eyelids completely shut	N	0	0	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
PROMINENCE OF EYE									
Normal	N	20	20	20	20	20	20	20	20
Exophthalmos	N	0	0	0	0	0	0	0	0
Enophthalmos	N	0	0	0	0	0	0	0	0

GROUP		I				II			
DOSAGE (MG/KG/DAY) <sup>a</sup> - ACRYLAMIDE		0(VEHICLE CONTROL)				50			
TEST		DOSAGE PERIOD				DOSAGE PERIOD			
		PRE-DOSAGE	DAY 1	DAY 9	DAY 17	PRE-DOSAGE	DAY 1	DAY 9	DAY 17
LACRIMATION									
(1) No excessive lacrimation	N	20	20	20	20	20	20	20	20
(2) Slight	N	0	0	0	0	0	0	0	0
(3) Severe	N	0	0	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
SALIVATION									
(1) No excessive salivation	N	20	20	20	20	20	20	20	20
(2) Wet margin along submaxillary area	N	0	0	0	0	0	0	0	0
(3) Severe	N	0	0	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
PILOERECTOR <sup>c</sup>	N	0	0	0	0	0	0	0	0
ABNORMAL RESPIRATION <sup>c</sup>	N	0	0	0	0	0	0	0	0
APPEARANCE									
(1) Clean and groomed	N	20	20	20	20	20	20	20	20
(2) Unkempt	N	0	0	0	0	0	0	0	0
(3) Stained by urine and feces	N	0	0	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
AROUSAL LEVEL									
(1) Very low (stuporous)	N	0	0	0	0	0	0	0	0
(2) Low (some exploratory movement)	N	0	0	0	1	0	0	0	0
(3) Apparently normal	N	20	20	20	19	20	20	20	20
(4) High (sudden darting and freezing)	N	0	0	0	0	0	0	0	0
(5) Very high (running and vocalization)	N	0	0	0	0	0	0	0	0
	MEDIAN SCORE	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
DEGREE OF MOTILITY									
(1) Apparently normal	N	20	20	20	20	20	20	20	0
(2) Moves with difficulty	N	0	0	0	0	0	0	0	20
(3) Unable to move	N	0	0	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0	1.0	2.0**
GAIT									
Normal	N	20	20	20	20	20	20	20	0**
Ataxic (swaying or lurching)	N	0	0	0	0	0	0	0	1
Exaggerated movements in limbs	N	0	0	0	0	0	0	0	19**
Tip-toe walk	N	0	0	0	0	0	0	0	0

GROUP		I				II			
DOSAGE (MG/KG/DAY) <sup>a</sup> - ACRYLAMIDE		0 (VEHICLE CONTROL)				50			
TEST		DOSAGE PERIOD				DOSAGE PERIOD			
		PRE-DOSAGE	DAY 1	DAY 9	DAY 17	PRE-DOSAGE	DAY 1	DAY 9	DAY 17
INVOLUNTARY BEHAVIOR <sup>d</sup>									
None apparent	N	20	20	20	20	20	20	20	20
Stereotyped behavior pattern	N	0	0	0	0	0	0	0	0
Bizarre behavior	N	0	0	0	0	0	0	0	0
Tremors in limbs	N	0	0	0	0	0	0	0	0
Whole body tremors or spasms	N	0	0	0	0	0	0	0	0
Unusual posture	N	0	0	0	0	0	0	0	0
Tonic-clonic seizure	N	0	0	0	0	0	0	0	0
FECAL BOLUSES	MEAN	0.7	1.2	1.0	1.3	0.5	0.8	1.0	1.2
	± S.D.	± 0.9	± 1.2	± 1.5	± 2.0	± 0.9	± 1.3	± 1.3	± 1.9
URINE POOLS	MEAN	0.6	1.0	0.8	0.9	0.5	0.6	0.9	1.2
	± S.D.	± 0.9	± 1.1	± 0.8	± 0.9	± 0.9	± 0.8	± 1.1	± 1.1
REACTION TO VISUAL STIMULUS									
(1) No reaction	N	6	6	6	8	5	8	8	4
(2) Moves toward or away from stimulus	N	14	14	14	12	15	12	12	10
(3) Startle response or freezing	N	0	0	0	0	0	0	0	6
(4) More energetic response	N	0	0	0	0	0	0	0	0
(5) Attacks or bites probe	N	0	0	0	0	0	0	0	0
	MEDIAN SCORE	2.0	2.50	2.0	2.0	2.0	2.0	2.0	2.0
REACTION TO TACTILE STIMULUS									
(1) No reaction	N	16	12	10	13	14	12	13	10
(2) Moves towards or away from stimulus	N	4	8	6	3	5	8	7	5
(3) Startle response or freezing	N	0	0	4	4	1	0	0	5
(4) More enrtgetic response	N	0	0	0	0	0	0	0	0
(5) Attacks or bites probe	N	0	0	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.5	1.0	1.0	1.0	1.0	1.0
REACTION TO AUDITORY STIMULUS									
(1) No reaction	N	0	0	0	1	2	2	0	2
(2) Moves towards or away from stimulus	N	1	2	2	0	1	0	1	1
(3) Startle response or freezing	N	19	18	17	19	15	18	19	16
(4) More energetic response	N	0	0	1	0	2	0	0	1
(5) Jumps at or away from the noise	N	0	0	0	0	0	0	0	0
	MEDIAN SCORE	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
REACTION TO TAIL PINCH									
(1) No reaction	N	4	9	4	6	5	5	2	3
(2) Moves toward or away from stimulus	N	9	7	10	7	11	12	7	7
(3) Startle response or freezing	N	3	4	4	7	3	3	10	7
(4) More energetic response	N	4	0	2	0	1	0	1	3
(5) Attacks or bites probe	N	0	0	0	0	0	0	0	0
	MEDIAN SCORE	2.0	2.0	2.0	2.0	2.0	2.0	3.0	2.50
PUPILLARY RESPONSE <sup>c</sup>	N	20	20	20	20	20	20	20	20

GROUP		I				II			
DOSAGE (MG/KG/DAY) <sup>a</sup> - ACRYLAMIDE		0 (VEHICLE CONTROL)				50			
TEST		DOSAGE PERIOD				DOSAGE PERIOD			
		PRE-DOSAGE	DAY 1	DAY 9	DAY 17	PRE-DOSAGE	DAY 1	DAY 9	DAY 17
AIR RIGHTING REACTION									
(1) Lands with all feed on the ground	N	20	16	16	18	20	19	20	17
(2) Uncoordinated landing	N	0	4	4	2	0	1	0	2
(3) Lands on back	N	0	0	0	0	0	0	0	1
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
VISUAL PLACING REACTION									
(1) Early extension of forelimbs	N	19	20	20	20	18	20	20	20
(2) Extension follows initial contact	N	1	0	0	0	2	0	0	0
(3) No extension after contact	N	0	0	0	0	0	0	0	0
	MEDIAN SCORE	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
MALES									
	N	10	10	10	10	10	10	10	10
FORELIMB GRIP STRENGTH (MAXIMUM g)	MEAN	631	688	821	792	632	704	715	805
	± S.D.	± 115	± 184	± 123	± 191	± 134	± 202	± 240	± 216
HINDLIMB GRIP STRENGTH (MAXIMUM g)	MEAN	800	882	916	936	763	914	886	882
	± S.D.	± 107	± 112	± 117	± 111	± 129	± 112	± 127	± 129
HINDLIMB FOOT SPREAD (AVERAGE cm)	MEAN	7.6	8.9	8.7	7.8	7.3	8.3	9.6	12.3
	± S.D.	± 1.7	± 2.6	± 2.3	± 1.9	± 2.0	± 2.5	± 1.8	± 1.7**
BODY WEIGHT (g)	MEAN	321	348	376	397	314	342	347	345
	± S.D.	± 55	± 54	± 52	± 51	± 52	± 51	± 41	± 35**
FEMALES									
	N	10	10	10	10	10	10	10	10
FORELIMB GRIP STRENGTH (MAXIMUM g)	MEAN	554	604	644	533	605	558	632	527
	± S.D.	± 184	± 174	± 196	± 118	± 226	± 204	± 159	± 172
HINDLIMB GRIP STRENGTH (MAXIMUM g)	MEAN	658	736	740	756	648	766	680	702
	± S.D.	± 99	± 92	± 192	± 164	± 160	± 106	± 130	± 168
FOOT SPREAD MEASURE (AVERAGE cm)	MEAN	7.0	8.2	7.2	7.4	7.0	7.5	9.6	11.7
	± S.D.	± 0.9	± 1.4	± 0.8	± 1.2	± 0.9	± 1.2	± 1.4**	± 1.1**
BODY WEIGHT (g)	MEAN	220	230	236	240	223	235	232	228
	± S.D.	± 50	± 47	± 43	± 43	± 50	± 45	± 34	± 30

DAY = DAY OF THE STUDY

(n) = Score assigned to graded test items.

a. Dosage occurred once every other day of the study, beginning on day 1 and ending on day 15 (total of eight dosages).

b. Observation made at the home cage.

c. Number of rats showing a response.

d. Observation made in the open field.

\* Significantly different from the vehicle control group value ( $P < 0.05$ ). For items showing the combined data for both males and females, the differences were significant for both sexes.

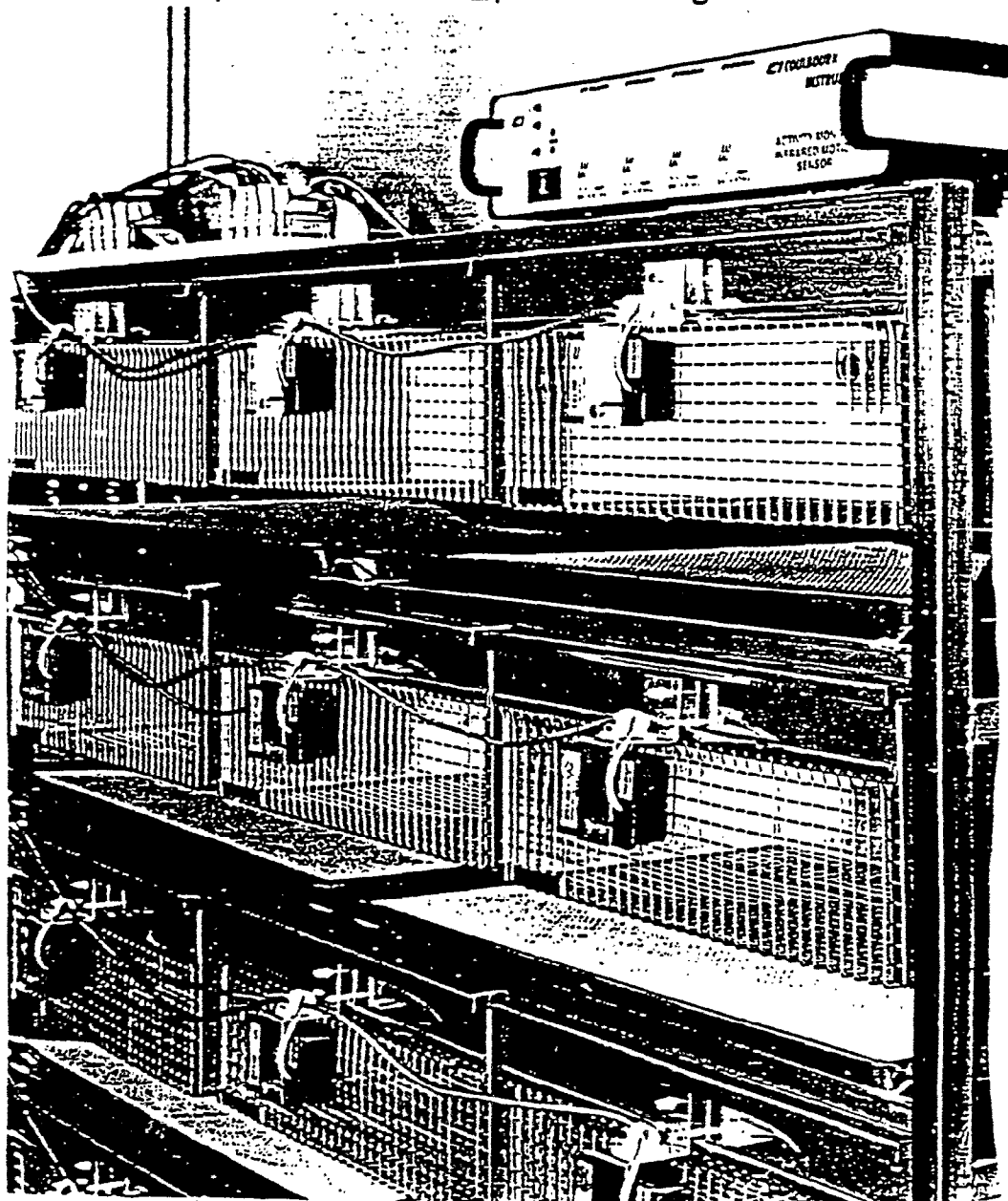
\*\* Significantly different from the vehicle control group value ( $P < 0.01$ ). For items showing the combined data for both males and females, the differences were significant for both sexes.

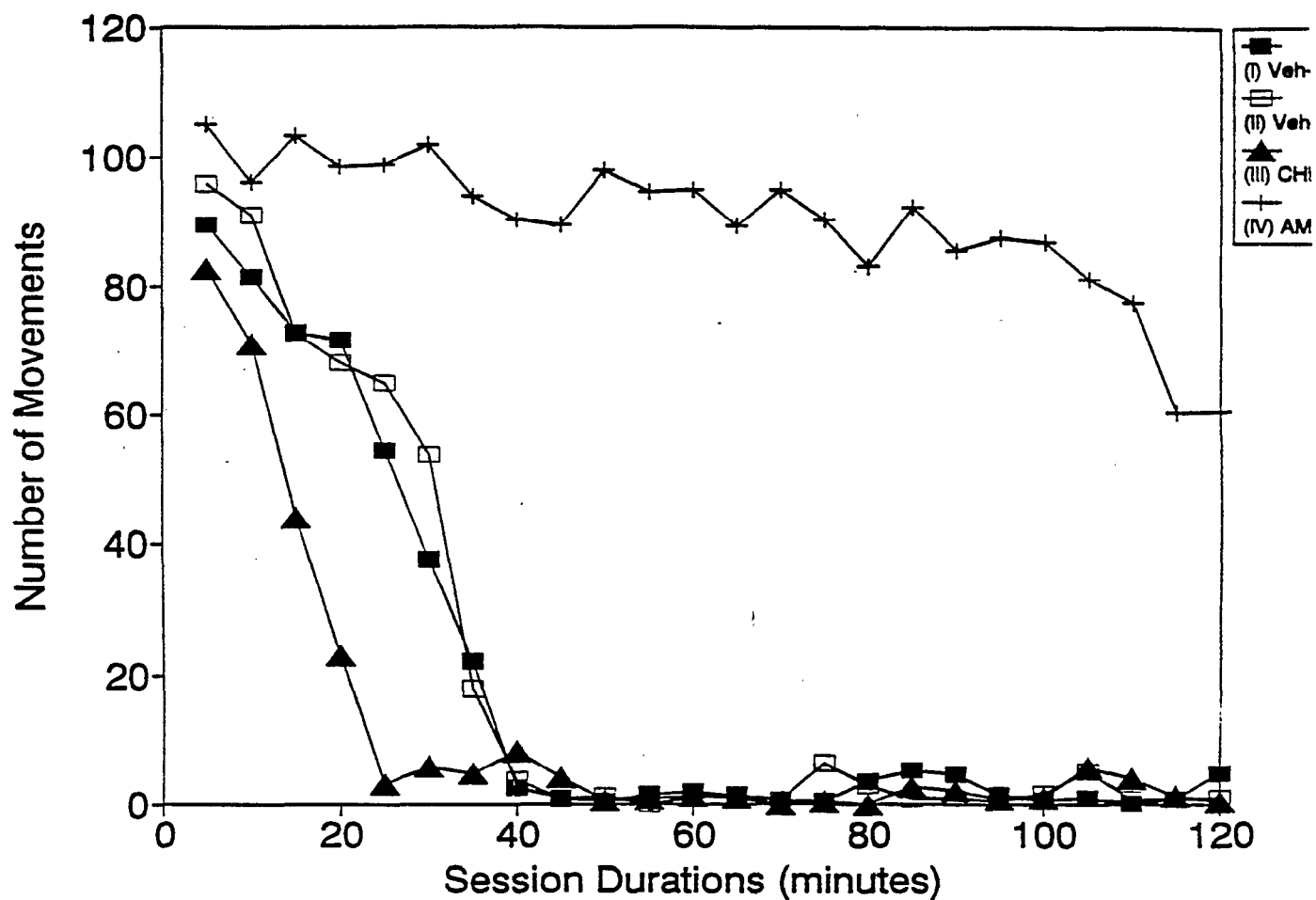
# Significantly different from the vehicle control group value ( $P < 0.05$ ) for one, but not both sexes (for the items showing the



## MOTOR ACTIVITY

Standard rat cages (double width) were used for the motor activity tests. The response of the passive infrared sensor was proportional to the extent of movement and was based on changes in the pattern of heat in the cage. Sets of eight sensors were connected to a distribution box from which a single cable led to the unit containing analog-to-digital (A/D) converters and a power supply; the unit could monitor 32 sensors. The A/D converters sampled the output of the sensors every 200 milliseconds under the control of a microcomputer. Using predefined thresholds, the computer classified the samples as representing no movement, a small movement, or a large movement. Two or more consecutive samples in the same movement category were defined as a discrete movement and the number of movements were summed over five minute blocks along with the total time spent in each category. A calibration device and associated software were used to standardize the output of the sensors prior to testing.





The data for the large and small movement categories were combined and then analyzed using a repeated measures analysis of variance with the probabilities corrected by the Greenhouse-Geiser procedure. The groups administered the test substances, amphetamine (AMP) and chlorpromazine (CHPZ), showed statistically significant differences from their respective controls in total number of movements (shown here) and time spent in movement as well as in the change throughout the session.

APPENDIX 6  
MOTOR ACTIVITY POSITIVE CONTROL #2

PROTOCOL 012-016:      MOTOR ACTIVITY EVALUATION IN Crl:CD®BR VAF/Plus® RATS  
ADMINISTERED CHLORPROMAZINE AND D-AMPHETAMINE (POSITIVE  
CONTROL STUDY)

A.      Methodology

1.      General Experimental Design and Procedures

The purpose of this study was to evaluate the effects of two test substances on motor activity. Data obtained in this study will be used for positive control information for validation of the Test Facility's Motor Activity Monitoring System in accordance with the testing guidelines of the U.S. Environmental Protection Agency (EPA FIFRA). The study was conducted in compliance with the Good Laboratory Practice Standards of the U.S. Environmental Protection Agency (EPA FIFRA/TSCA).

The test substances, chlorpromazine<sup>a</sup> and d-amphetamine<sup>a</sup>, were administered to fifteen male and fifteen female rats. Dosages of 0 (Vehicle, 0.9% Sodium Chloride for Injection, USP), 1, 2 and 4 mg/kg/day of chlorpromazine were administered intraperitoneally approximately 70 minutes before an evaluation of motor activity in a two-hour test session. Dosages of 0 (Vehicle, 0.9% Sodium Chloride for Injection, USP), 0.50, 1 and 4 mg/kg/day of d-amphetamine sulfate were administered intraperitoneally immediately before the same evaluation of motor activity. The dosage volume was 1 mL/kg, based on the individual body weights recorded on the day of dosage administration.

-----  
a.      The test substances were considered 100% pure for purposes of dosage calculations.

Dosage and motor activity testing occurred on days 1, 3, 6, 8, 10, 13, 15 and 17 of the study period. The study was a repeated-measures design in which each rat received all dosages of both test substances. At least one day on which no testing occurred separated each day of administration. The sequence of administration of the test substances and vehicle varied across rats.

## 2. Conduct of Study

The in-life phase of the study occurred as follows:

Acclimation Period.	04/08/92 - 04/21/92
Administration Period.	04/22/92 - 05/08/92
1st Dosage/Motor Activity Test.	04/22/92
2nd Dosage/Motor Activity Test.	04/24/92
3rd Dosage/Motor Activity Test.	04/27/92
4th Dosage/Motor Activity Test.	04/29/92
5th Dosage/Motor Activity Test.	05/01/92
6th Dosage/Motor Activity Test.	05/04/92
7th Dosage/Motor Activity Test.	05/06/92
8th Dosage/Motor Activity Test.	05/08/92
Scheduled Sacrifice.	05/14/92

## 3. Sponsor

This study was sponsored by the Test Facility: Argus Research Laboratories, Inc., 905 Sheehy Drive, Horsham, Pennsylvania 19044. The Sponsor's Representative was Alan M. Hoberman, Ph.D., D.A.B.T. (Director of Research).

## 4. Test Facility, Study Director and Participating Personnel

The study was conducted by and at Argus Research Laboratories, Inc., 905 Sheehy Drive, Horsham, Pennsylvania 19044. The Study Director was John A. Foss, Ph.D. (Group Leader, Neurotoxicology).

Key personnel participating in the conduct of the study were John F. Barnett, B.S. (Director of Laboratory Operation), Ronald J. McCarty, B.S. (Assistant Director of Laboratory Operations), Margaret M. Martin (Research

Assistant/Assistant Supervisor), Susan L. DeHaven, B.S. (Laboratory Associate), Karen S. Willey (Animal Care Technician), James W. Bray, B.A. (Senior Information Specialist), Cheryl L. Van, B.A. (Methods Writer) and Lynne C. Rapson (Administrative Coordinator). Additional personnel participating in the conduct of the study are identified in the raw data.

Curricula vitae and training records of personnel involved in the study are on file at Argus Research Laboratories, Inc.

5. Test Substance and Vehicle Identification

Chlorpromazine hydrochloride (Lot 50H0659, received April 20, 1992) and d-amphetamine sulfate (Lots 020H0676 and 41H0145, received April 20, 1992), both white powders, were purchased from Sigma Chemical Co. (St. Louis, Missouri). Documentation of the method of synthesis, purity and characterization, and stability of the test substances is available in the Supplier's records. The test substances were stored at room temperature and protected from light.

The vehicle 0.9% Sodium Chloride for Injection, USP (Lot J1C049B) was received from McGaw, Inc. on October 16, 1991. It was stored at room temperature.

B. Animal Data

1. Test System

The Charles River Crl:CD®BR VAF/Plus® (Sprague-Dawley) rat was selected for evaluation of the test substances because this strain has been widely used throughout industry for nonclinical studies of toxicity. The rats were selected from adult rats in the Test Facility general population or were received from Charles River Laboratories, Inc. The following summarizes the information available regarding the rats selected for study.

Male Rats

	<u>Number Used</u>	<u>Approximate Date of Birth</u>	<u>Arrival Date</u>	<u>Approximate Age at Arrival</u>	<u>Weight on Day 2 of Acclimation</u>
Born at Test Facility	10	02/02/92 <sup>a</sup>	02/02/92 <sup>a</sup>	65 days	331 - 498 g
General Population	4	b	b	65 days	340 - 392 g
Received from Charles River Laboratories	1	01/21/92	03/10/92	50 days	353 g

Female Rats

	<u>Number Used</u>	<u>Approximate Date of Birth</u>	<u>Arrival Date</u>	<u>Approximate Age at arrival</u>	<u>Weight on Day 2 of Acclimation</u>
Born at Test Facility	4	02/02/92	02/02/92	65 days	241 - 297 g
Received from Charles River Laboratories	11	01/13/92 and 01/20/92	03/10/92 and 03/24/92	58-65 days	231 - 324 g

All rats were selected from the Test Facility General population which was derived from rats received from Charles River Laboratories. On day 2 of acclimation, the male rats weighed between 331 g - 498 g and the female rats weighed between 231 g - 324 g. Based on these weights, all rats were estimated to be approximately 65 days of age.

2. Experimental Design and Control of Bias

The male and female rats were assigned to individual housing on the basis of a computer-generated randomization procedure. At this time, a temporary

- 
- a. Rats were born at the Test Facility. General population arrival date equals date of birth. No arrival weight is available.
  - b. Rats were selected from the Test Facility general population. No arrival date or date of birth information is available.

PROTOCOL 012-016: MOTOR ACTIVITY EVALUATION IN Cr1:CD(TM)BR VAF/Plus(TM) RATS ADMINISTERED CHLORPROMAZINE AND D-AMPHETAMINE (POSITIVE CONTROL STUDY)

TABLE 1 (PAGE 1): MOTOR ACTIVITY - SUMMARY - MALE RATS

DOSAGE CHLORPROMAZINE (MG/KG/DAY)		I 0(VEHICLE)	II 1.0	III 2.0	IV 4.0
NUMBER OF RATS	N	15	15	15	15
NUMBER OF MOVEMENTS					
BLOCK 1	MEAN ± S.D.	71.3 ± 11.0	72.7 ± 11.0	69.9 ± 11.9	60.4 ± 15.0
BLOCK 2	MEAN ± S.D.	75.1 ± 13.8	65.0 ± 20.2	56.4 ± 17.6	37.3 ± 25.4
BLOCK 3	MEAN ± S.D.	64.9 ± 14.9	58.5 ± 18.2	48.4 ± 23.0	28.9 ± 23.9
BLOCK 4	MEAN ± S.D.	61.7 ± 13.3	51.3 ± 22.7	37.8 ± 33.4	29.2 ± 21.8
BLOCK 5	MEAN ± S.D.	59.7 ± 24.7	46.5 ± 25.2	27.3 ± 29.8	23.2 ± 23.3
BLOCK 6	MEAN ± S.D.	53.7 ± 21.1	44.1 ± 26.8	22.2 ± 26.6	15.9 ± 21.0
BLOCK 7	MEAN ± S.D.	37.4 ± 27.9	33.6 ± 24.7	16.5 ± 22.7	9.9 ± 16.8
BLOCK 8	MEAN ± S.D.	32.3 ± 25.5	35.2 ± 28.4	10.3 ± 13.3	10.1 ± 19.4
BLOCK 9	MEAN ± S.D.	25.1 ± 28.6	25.5 ± 25.1	15.7 ± 25.1	9.4 ± 11.8
BLOCK 10	MEAN ± S.D.	18.1 ± 26.9	23.5 ± 23.6	9.6 ± 18.2	7.5 ± 14.0
BLOCK 11	MEAN ± S.D.	15.2 ± 24.4	13.2 ± 19.0	11.1 ± 18.3	2.7 ± 6.6
BLOCK 12	MEAN ± S.D.	20.7 ± 30.5	12.1 ± 21.8	6.1 ± 15.1	6.3 ± 8.0
BLOCK 13	MEAN ± S.D.	17.1 ± 27.1	5.3 ± 15.3	4.9 ± 8.5	3.5 ± 7.6
BLOCK 14	MEAN ± S.D.	15.8 ± 28.0	1.4 ± 3.3	6.1 ± 16.4	5.6 ± 9.9
BLOCK 15	MEAN ± S.D.	7.5 ± 14.5	1.1 ± 1.5	4.3 ± 7.4	8.0 ± 13.7
BLOCK 16	MEAN ± S.D.	9.7 ± 18.1	1.9 ± 4.2	4.5 ± 6.2	11.5 ± 16.9
BLOCK 17	MEAN ± S.D.	8.2 ± 17.3	1.5 ± 2.8	5.7 ± 15.3	3.7 ± 6.2
BLOCK 18	MEAN ± S.D.	7.1 ± 17.2	1.7 ± 2.2	4.1 ± 7.6	4.3 ± 8.5
BLOCK 19	MEAN ± S.D.	3.1 ± 5.2	2.5 ± 4.3	3.3 ± 10.2	7.5 ± 18.4
BLOCK 20	MEAN ± S.D.	5.1 ± 8.5	1.4 ± 2.9	2.7 ± 6.6	5.1 ± 8.8
BLOCK 21	MEAN ± S.D.	3.3 ± 9.7	6.8 ± 10.8	0.8 ± 1.3	10.4 ± 16.4
BLOCK 22	MEAN ± S.D.	5.7 ± 11.6	1.5 ± 4.6	0.9 ± 1.1	3.7 ± 7.4
BLOCK 23	MEAN ± S.D.	8.3 ± 22.3	1.7 ± 1.9	2.7 ± 6.8	0.9 ± 1.7
BLOCK 24	MEAN ± S.D.	1.5 ± 2.0	1.9 ± 1.9	1.8 ± 4.3	3.3 ± 6.6
TOTAL	MEAN ± S.D.	627.5 ± 267.0	509.9 ± 190.9	372.8 ± 168.8**	308.3 ± 191.0**

STATISTICAL ANALYSES OF MOTOR ACTIVITY WERE RESTRICTED TO THE TOTAL NUMBER OF MOVEMENTS AND TOTAL TIME SPENT IN MOVEMENT.  
TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

\*\* Significantly different from the vehicle control group TOTAL ( $P \leq 0.01$ ).



PROTOCOL 012-016: MOTOR ACTIVITY EVALUATION IN Crl:CD(TM)BR VAF/Plus(TM) RATS ADMINISTERED CHLORPROMAZINE AND D-AMPHETAMINE (POSITIVE CONTROL STUDY)

TABLE 1 (PAGE 2): MOTOR ACTIVITY - SUMMARY - MALE RATS

DOSAGE CHLORPROMAZINE (MG/KG/DAY)		I 0(VEHICLE)	II 1.0	III 2.0	IV 4.0
NUMBER OF RATS	N	15	15	15	15
TIME (SECONDS) SPENT IN MOVEMENT					
BLOCK 1	MEAN ± S.D.	163.2 ± 39.0	152.8 ± 42.4	124.3 ± 33.8	99.1 ± 44.1
BLOCK 2	MEAN ± S.D.	149.7 ± 33.5	135.0 ± 59.2	100.1 ± 46.3	57.9 ± 50.3
BLOCK 3	MEAN ± S.D.	125.3 ± 38.9	108.4 ± 52.2	76.8 ± 54.1	36.8 ± 36.4
BLOCK 4	MEAN ± S.D.	115.7 ± 50.1	84.7 ± 41.2	51.9 ± 51.8	36.6 ± 37.9
BLOCK 5	MEAN ± S.D.	94.1 ± 48.7	73.7 ± 50.4	34.9 ± 40.4	29.3 ± 33.2
BLOCK 6	MEAN ± S.D.	82.0 ± 45.4	72.8 ± 58.8	26.3 ± 40.0	15.3 ± 25.5
BLOCK 7	MEAN ± S.D.	53.1 ± 45.7	49.0 ± 40.3	18.5 ± 26.1	10.3 ± 21.5
BLOCK 8	MEAN ± S.D.	39.7 ± 42.6	58.4 ± 59.2	9.9 ± 15.9	10.0 ± 27.6
BLOCK 9	MEAN ± S.D.	39.8 ± 54.3	35.4 ± 41.1	21.7 ± 40.6	7.5 ± 10.7
BLOCK 10	MEAN ± S.D.	38.9 ± 71.9	38.2 ± 45.8	11.5 ± 30.2	7.1 ± 16.2
BLOCK 11	MEAN ± S.D.	17.1 ± 30.8	17.8 ± 32.6	11.5 ± 24.2	1.3 ± 5.2
BLOCK 12	MEAN ± S.D.	36.0 ± 54.4	14.2 ± 27.9	4.6 ± 12.2	4.3 ± 7.4
BLOCK 13	MEAN ± S.D.	28.1 ± 51.6	6.2 ± 20.8	3.8 ± 8.7	2.3 ± 6.2
BLOCK 14	MEAN ± S.D.	15.9 ± 30.3	1.4 ± 4.4	6.3 ± 19.6	4.1 ± 10.1
BLOCK 15	MEAN ± S.D.	7.6 ± 18.4	0.3 ± 0.4	3.6 ± 8.6	5.9 ± 13.3
BLOCK 16	MEAN ± S.D.	11.1 ± 26.3	1.5 ± 3.8	3.4 ± 8.1	15.2 ± 30.0
BLOCK 17	MEAN ± S.D.	10.1 ± 28.0	0.9 ± 1.9	4.3 ± 13.8	2.2 ± 5.6
BLOCK 18	MEAN ± S.D.	5.9 ± 14.5	0.9 ± 1.6	2.6 ± 6.2	3.9 ± 10.8
BLOCK 19	MEAN ± S.D.	3.1 ± 7.6	1.4 ± 2.5	3.4 ± 11.8	7.9 ± 25.2
BLOCK 20	MEAN ± S.D.	3.7 ± 8.6	0.5 ± 1.3	1.3 ± 3.1	2.9 ± 6.3
BLOCK 21	MEAN ± S.D.	3.4 ± 12.4	7.1 ± 13.1	0.2 ± 0.4	11.9 ± 24.0
BLOCK 22	MEAN ± S.D.	6.7 ± 17.5	1.5 ± 5.9	0.3 ± 0.6	2.5 ± 5.5
BLOCK 23	MEAN ± S.D.	7.8 ± 24.6	0.3 ± 0.7	2.1 ± 7.2	0.1 ± 0.5
BLOCK 24	MEAN ± S.D.	0.7 ± 1.0	0.7 ± 1.1	0.9 ± 3.1	2.2 ± 5.9
TOTAL	MEAN ± S.D.	1058.8 ± 520.0	862.9 ± 391.0	524.1 ± 281.7**	376.7 ± 288.2**

STATISTICAL ANALYSES OF MOTOR ACTIVITY WERE RESTRICTED TO THE TOTAL NUMBER OF MOVEMENTS AND TOTAL TIME SPENT IN MOVEMENT.  
TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

\*\* Significantly different from the vehicle control group TOTAL ( $p < 0.01$ ).

PROTOCOL 012-016: MOTOR ACTIVITY EVALUATION IN Cr1:CD(TM)BR VAF/Plus(TM) RATS ADMINISTERED CHLORPROMAZINE AND D-AMPHETAMINE (POSITIVE CONTROL STUDY)

TABLE 1 (PAGE 1): MOTOR ACTIVITY - SUMMARY - FEMALE RATS

DOSAGE CHLORPROMAZINE (MG/KG/DAY)		I 0(VEHICLE)	II 1.0	III 2.0	IV 4.0
NUMBER OF RATS	N	15	15	15	15
NUMBER OF MOVEMENTS					
BLOCK 1	MEAN ± S.D.	73.9 ± 9.1	73.1 ± 8.2	70.1 ± 9.6	60.8 ± 13.0
BLOCK 2	MEAN ± S.D.	77.7 ± 7.9	69.5 ± 14.8	68.7 ± 16.1	53.5 ± 17.4
BLOCK 3	MEAN ± S.D.	70.3 ± 11.5	58.2 ± 20.0	49.5 ± 23.3	40.3 ± 21.6
BLOCK 4	MEAN ± S.D.	70.3 ± 11.6	52.9 ± 23.5	47.0 ± 19.5	23.8 ± 15.7
BLOCK 5	MEAN ± S.D.	56.1 ± 15.5	42.5 ± 23.9	28.7 ± 21.5	12.0 ± 13.5
BLOCK 6	MEAN ± S.D.	40.9 ± 31.1	36.1 ± 27.0	23.7 ± 30.0	4.3 ± 10.5
BLOCK 7	MEAN ± S.D.	23.9 ± 24.0	28.3 ± 28.2	15.4 ± 24.8	2.3 ± 4.3
BLOCK 8	MEAN ± S.D.	28.2 ± 30.1	16.5 ± 21.9	8.9 ± 17.0	3.5 ± 11.3
BLOCK 9	MEAN ± S.D.	18.8 ± 25.0	7.1 ± 14.4	3.9 ± 6.8	4.4 ± 9.0
BLOCK 10	MEAN ± S.D.	10.0 ± 14.2	7.0 ± 16.5	3.8 ± 14.2	2.5 ± 5.0
BLOCK 11	MEAN ± S.D.	10.8 ± 17.0	5.3 ± 15.5	3.9 ± 12.6	1.7 ± 3.6
BLOCK 12	MEAN ± S.D.	11.1 ± 16.9	2.4 ± 5.3	5.1 ± 8.7	4.3 ± 9.6
BLOCK 13	MEAN ± S.D.	10.0 ± 17.6	2.7 ± 4.0	4.2 ± 8.5	1.3 ± 2.6
BLOCK 14	MEAN ± S.D.	8.6 ± 18.5	5.0 ± 8.0	2.4 ± 3.1	2.8 ± 4.4
BLOCK 15	MEAN ± S.D.	12.3 ± 22.0	3.2 ± 6.6	0.9 ± 1.8	1.2 ± 2.1
BLOCK 16	MEAN ± S.D.	16.1 ± 23.1	4.6 ± 10.8	2.1 ± 5.1	2.1 ± 4.6
BLOCK 17	MEAN ± S.D.	12.2 ± 17.3	2.5 ± 4.0	1.3 ± 2.3	3.4 ± 6.4
BLOCK 18	MEAN ± S.D.	15.7 ± 17.8	1.3 ± 1.5	1.1 ± 2.2	1.5 ± 2.1
BLOCK 19	MEAN ± S.D.	8.5 ± 16.9	1.5 ± 1.6	1.7 ± 5.1	2.7 ± 7.1
BLOCK 20	MEAN ± S.D.	7.5 ± 15.1	5.4 ± 13.9	2.3 ± 7.8	1.7 ± 4.0
BLOCK 21	MEAN ± S.D.	6.0 ± 19.7	3.4 ± 8.1	5.8 ± 10.3	3.5 ± 9.2
BLOCK 22	MEAN ± S.D.	5.2 ± 15.0	5.3 ± 8.4	1.8 ± 2.9	3.3 ± 7.7
BLOCK 23	MEAN ± S.D.	3.6 ± 7.7	1.7 ± 1.8	2.1 ± 3.0	4.1 ± 11.2
BLOCK 24	MEAN ± S.D.	1.3 ± 1.6	4.5 ± 8.9	1.5 ± 2.1	4.3 ± 12.2
TOTAL	MEAN ± S.D.	598.9 ± 199.6	440.2 ± 150.0*	355.9 ± 135.8**	245.3 ± 75.8**

STATISTICAL ANALYSES OF MOTOR ACTIVITY WERE RESTRICTED TO THE TOTAL NUMBER OF MOVEMENTS AND TOTAL TIME SPENT IN MOVEMENT.

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

\* Significantly different from the vehicle control group TOTAL ( $P \leq 0.05$ ).

\*\* Significantly different from the vehicle control group TOTAL ( $P \leq 0.01$ ).

PROTOCOL 012-016: MOTOR ACTIVITY EVALUATION IN Crl:CD(TM)BR VAF/Plus(TM) RATS ADMINISTERED CHLORPROMAZINE AND D-AMPHETAMINE (POSITIVE CONTROL STUDY)

TABLE 1 (PAGE 2): MOTOR ACTIVITY - SUMMARY - FEMALE RATS

DOSAGE CHLORPROMAZINE (MG/KG/DAY)		I 0(VEHICLE)	II 1.0	III 2.0	IV 4.0
NUMBER OF RATS	N	15	15	15	15
TIME (SECONDS) SPENT IN MOVEMENT					
BLOCK 1	MEAN ± S.D.	156.3 ± 36.9	147.3 ± 34.8	143.0 ± 37.7	102.6 ± 39.5
BLOCK 2	MEAN ± S.D.	144.9 ± 26.6	121.1 ± 39.2	122.3 ± 39.5	85.1 ± 39.2
BLOCK 3	MEAN ± S.D.	125.5 ± 36.2	94.9 ± 45.3	86.8 ± 55.3	52.9 ± 43.4
BLOCK 4	MEAN ± S.D.	107.7 ± 29.0	90.4 ± 50.8	66.7 ± 46.2	24.5 ± 20.5
BLOCK 5	MEAN ± S.D.	82.0 ± 44.3	60.9 ± 43.7	34.9 ± 38.0	11.8 ± 21.3
BLOCK 6	MEAN ± S.D.	65.1 ± 58.8	50.9 ± 52.0	33.7 ± 50.4	3.5 ± 12.1
BLOCK 7	MEAN ± S.D.	32.1 ± 44.3	45.6 ± 58.6	25.4 ± 49.7	1.9 ± 4.5
BLOCK 8	MEAN ± S.D.	38.8 ± 49.5	18.9 ± 31.4	8.5 ± 17.9	4.2 ± 15.2
BLOCK 9	MEAN ± S.D.	23.1 ± 34.0	10.1 ± 21.7	1.8 ± 3.8	3.0 ± 7.4
BLOCK 10	MEAN ± S.D.	9.4 ± 22.5	9.5 ± 25.4	12.0 ± 46.5	3.0 ± 8.2
BLOCK 11	MEAN ± S.D.	7.8 ± 14.0	4.7 ± 16.0	3.5 ± 12.9	0.6 ± 1.7
BLOCK 12	MEAN ± S.D.	11.7 ± 21.4	1.3 ± 4.4	3.3 ± 6.9	4.3 ± 11.2
BLOCK 13	MEAN ± S.D.	8.7 ± 19.4	1.9 ± 4.0	3.1 ± 7.9	0.7 ± 2.0
BLOCK 14	MEAN ± S.D.	8.9 ± 20.7	3.1 ± 6.2	1.0 ± 1.8	1.4 ± 2.9
BLOCK 15	MEAN ± S.D.	15.5 ± 36.2	1.9 ± 4.6	0.3 ± 0.7	0.6 ± 1.6
BLOCK 16	MEAN ± S.D.	20.0 ± 34.7	3.4 ± 9.6	0.9 ± 2.8	1.1 ± 2.5
BLOCK 17	MEAN ± S.D.	8.5 ± 14.6	1.0 ± 2.0	0.5 ± 1.3	1.9 ± 4.3
BLOCK 18	MEAN ± S.D.	17.1 ± 25.5	0.5 ± 0.8	0.5 ± 1.4	0.7 ± 1.1
BLOCK 19	MEAN ± S.D.	8.6 ± 20.8	0.6 ± 1.2	2.1 ± 7.5	2.1 ± 6.9
BLOCK 20	MEAN ± S.D.	6.1 ± 16.1	6.0 ± 21.0	2.1 ± 7.5	1.6 ± 4.5
BLOCK 21	MEAN ± S.D.	8.8 ± 33.8	3.2 ± 10.8	4.4 ± 10.0	2.6 ± 7.5
BLOCK 22	MEAN ± S.D.	4.7 ± 16.2	5.4 ± 11.9	0.9 ± 2.6	2.7 ± 6.8
BLOCK 23	MEAN ± S.D.	2.5 ± 7.2	0.6 ± 1.0	0.9 ± 1.8	5.1 ± 14.0
BLOCK 24	MEAN ± S.D.	0.5 ± 1.1	2.7 ± 5.9	1.3 ± 3.3	5.5 ± 17.7
TOTAL	MEAN ± S.D.	914.3 ± 343.6	685.8 ± 315.0*	559.9 ± 294.8**	323.3 ± 154.6**

STATISTICAL ANALYSES OF MOTOR ACTIVITY WERE RESTRICTED TO THE TOTAL NUMBER OF MOVEMENTS AND TOTAL TIME SPENT IN MOVEMENT.  
TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

\* Significantly different from the vehicle control group TOTAL ( $P \leq 0.05$ ).

\*\* Significantly different from the vehicle control group TOTAL ( $P \leq 0.01$ ).

PROTOCOL 012-016: MOTOR ACTIVITY EVALUATION IN Crl:CD(TM)BR VAF/Plus(TM) RATS ADMINISTERED CHLORPROMAZINE AND D-AMPHETAMINE (POSITIVE CONTROL STUDY)

TABLE 1 (PAGE 1): MOTOR ACTIVITY - SUMMARY - MALE RATS

DOSAGE D-AMPHETAMINE (MG/KG/DAY)		V 0(VEHICLE)	VI 0.5	VII 1.0	VIII 4.0
NUMBER OF RATS	N	15	15	15	15
NUMBER OF MOVEMENTS					
BLOCK 1	MEAN ± S.D.	77.7 ± 7.2	72.6 ± 10.4	71.7 ± 15.0	77.1 ± 15.6
BLOCK 2	MEAN ± S.D.	73.9 ± 9.8	74.8 ± 11.5	71.0 ± 12.8	72.1 ± 14.4
BLOCK 3	MEAN ± S.D.	73.5 ± 9.1	74.5 ± 9.1	76.3 ± 13.9	62.9 ± 28.4
BLOCK 4	MEAN ± S.D.	64.0 ± 17.8	77.8 ± 8.8	76.3 ± 12.1	58.2 ± 28.8
BLOCK 5	MEAN ± S.D.	61.5 ± 18.0	75.2 ± 9.7	77.9 ± 9.0	51.3 ± 30.0
BLOCK 6	MEAN ± S.D.	49.0 ± 26.2	79.1 ± 10.6	75.1 ± 11.1	53.5 ± 27.7
BLOCK 7	MEAN ± S.D.	41.6 ± 31.1	74.7 ± 11.0	75.1 ± 15.6	45.8 ± 33.4
BLOCK 8	MEAN ± S.D.	33.9 ± 32.8	71.4 ± 16.9	74.8 ± 12.1	46.7 ± 37.9
BLOCK 9	MEAN ± S.D.	25.0 ± 29.5	68.3 ± 12.8	76.7 ± 14.0	48.0 ± 37.1
BLOCK 10	MEAN ± S.D.	24.5 ± 30.2	64.1 ± 13.0	72.5 ± 10.8	49.3 ± 42.4
BLOCK 11	MEAN ± S.D.	22.9 ± 27.2	63.1 ± 19.2	74.6 ± 18.1	51.2 ± 35.4
BLOCK 12	MEAN ± S.D.	19.2 ± 24.3	62.6 ± 19.4	69.7 ± 14.8	48.3 ± 35.5
BLOCK 13	MEAN ± S.D.	13.0 ± 18.8	60.8 ± 21.7	73.7 ± 11.8	46.7 ± 35.1
BLOCK 14	MEAN ± S.D.	5.6 ± 12.0	58.0 ± 24.9	75.0 ± 15.7	52.1 ± 29.4
BLOCK 15	MEAN ± S.D.	4.1 ± 9.8	55.0 ± 22.0	74.0 ± 17.4	50.7 ± 31.3
BLOCK 16	MEAN ± S.D.	6.2 ± 10.5	51.7 ± 24.3	74.7 ± 15.9	54.7 ± 35.6
BLOCK 17	MEAN ± S.D.	4.5 ± 8.4	47.3 ± 24.2	71.4 ± 16.1	43.1 ± 28.8
BLOCK 18	MEAN ± S.D.	4.7 ± 10.1	41.9 ± 29.6	67.7 ± 10.7	44.8 ± 25.7
BLOCK 19	MEAN ± S.D.	7.2 ± 12.4	40.5 ± 31.9	63.3 ± 18.2	47.4 ± 25.4
BLOCK 20	MEAN ± S.D.	3.2 ± 4.3	34.9 ± 29.9	59.1 ± 22.2	47.1 ± 24.5
BLOCK 21	MEAN ± S.D.	4.3 ± 8.8	33.5 ± 27.8	57.3 ± 28.5	58.4 ± 23.6
BLOCK 22	MEAN ± S.D.	4.1 ± 7.2	27.3 ± 30.2	57.5 ± 25.8	53.9 ± 25.2
BLOCK 23	MEAN ± S.D.	2.3 ± 2.5	24.0 ± 29.0	55.8 ± 27.9	57.6 ± 28.4
BLOCK 24	MEAN ± S.D.	2.7 ± 3.9	24.6 ± 29.0	50.1 ± 29.0	55.1 ± 25.2
TOTAL	MEAN ± S.D.	628.7 ± 182.7	1357.7 ± 292.7**	1671.4 ± 253.4**	1276.0 ± 511.0**

STATISTICAL ANALYSES OF MOTOR ACTIVITY WERE RESTRICTED TO THE TOTAL NUMBER OF MOVEMENTS AND TOTAL TIME SPENT IN MOVEMENT.

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

\*\* Significantly different from the vehicle control group TOTAL ( $P \leq 0.01$ ).

PROTOCOL 012-016: MOTOR ACTIVITY EVALUATION IN Crl:CD(TM)BR VAF/Plus(TM) RATS ADMINISTERED CHLORPROMAZINE  
AND D-AMPHETAMINE (POSITIVE CONTROL STUDY)

TABLE 1 (PAGE 2): MOTOR ACTIVITY - SUMMARY - MALE RATS

DOSAGE D-AMPHETAMINE (MG/KG/DAY)		V 0(VEHICLE)	VI 0.5	VII 1.0	VIII 4.0
NUMBER OF RATS	N	15	15	15	15
TIME (SECONDS) SPENT IN MOVEMENT					
BLOCK 1	MEAN ± S.D.	167.3 ± 37.4	193.0 ± 39.1	210.2 ± 36.6	190.7 ± 42.1
BLOCK 2	MEAN ± S.D.	145.5 ± 29.4	191.5 ± 33.8	206.7 ± 35.9	155.3 ± 73.9
BLOCK 3	MEAN ± S.D.	128.0 ± 34.7	185.5 ± 31.7	184.1 ± 32.7	131.4 ± 90.0
BLOCK 4	MEAN ± S.D.	107.9 ± 45.4	182.9 ± 30.0	180.5 ± 43.9	113.0 ± 89.3
BLOCK 5	MEAN ± S.D.	101.5 ± 48.0	167.5 ± 34.3	186.9 ± 34.7	102.7 ± 100.5
BLOCK 6	MEAN ± S.D.	79.3 ± 53.7	154.4 ± 44.3	176.5 ± 45.4	104.5 ± 101.0
BLOCK 7	MEAN ± S.D.	62.7 ± 52.5	149.1 ± 39.7	178.5 ± 47.5	113.6 ± 113.4
BLOCK 8	MEAN ± S.D.	48.3 ± 56.4	140.2 ± 47.3	187.3 ± 49.6	105.3 ± 107.6
BLOCK 9	MEAN ± S.D.	35.3 ± 46.2	134.9 ± 49.3	172.5 ± 44.0	103.5 ± 105.9
BLOCK 10	MEAN ± S.D.	50.5 ± 72.8	133.9 ± 55.5	179.4 ± 34.7	111.1 ± 106.0
BLOCK 11	MEAN ± S.D.	32.3 ± 49.5	114.1 ± 56.9	162.1 ± 47.6	107.6 ± 98.2
BLOCK 12	MEAN ± S.D.	24.3 ± 35.0	116.1 ± 51.6	158.0 ± 48.9	96.7 ± 102.6
BLOCK 13	MEAN ± S.D.	14.9 ± 27.8	106.6 ± 56.0	148.0 ± 46.5	85.3 ± 91.4
BLOCK 14	MEAN ± S.D.	6.2 ± 15.7	93.9 ± 51.6	155.1 ± 39.2	91.9 ± 90.6
BLOCK 15	MEAN ± S.D.	4.5 ± 14.4	94.0 ± 52.3	135.7 ± 36.9	82.5 ± 87.8
BLOCK 16	MEAN ± S.D.	5.7 ± 14.6	76.9 ± 53.4	141.1 ± 34.0	91.0 ± 91.3
BLOCK 17	MEAN ± S.D.	5.4 ± 14.8	67.8 ± 44.4	121.9 ± 41.6	78.2 ± 95.8
BLOCK 18	MEAN ± S.D.	4.3 ± 12.7	63.6 ± 55.5	127.1 ± 39.6	75.4 ± 88.0
BLOCK 19	MEAN ± S.D.	7.7 ± 21.7	60.7 ± 55.5	102.9 ± 35.8	77.1 ± 87.5
BLOCK 20	MEAN ± S.D.	2.3 ± 3.9	51.4 ± 48.4	101.2 ± 52.7	73.2 ± 78.4
BLOCK 21	MEAN ± S.D.	4.6 ± 11.2	61.5 ± 71.6	97.5 ± 65.5	90.8 ± 73.1
BLOCK 22	MEAN ± S.D.	3.1 ± 6.8	50.3 ± 60.8	103.6 ± 55.9	88.2 ± 78.6
BLOCK 23	MEAN ± S.D.	1.1 ± 1.8	34.3 ± 48.5	107.8 ± 70.1	96.9 ± 76.2
BLOCK 24	MEAN ± S.D.	2.2 ± 3.6	36.7 ± 47.8	91.9 ± 70.8	104.4 ± 73.1
TOTAL	MEAN ± S.D.	1044.9 ± 394.4	2660.7 ± 765.3**	3616.5 ± 661.7**	2470.5 ± 1705.6**

STATISTICAL ANALYSES OF MOTOR ACTIVITY WERE RESTRICTED TO THE TOTAL NUMBER OF MOVEMENTS AND TOTAL TIME SPENT IN MOVEMENT.  
TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

\*\* Significantly different from the vehicle control group TOTAL ( $P \leq 0.01$ ).

PROTOCOL 012-016: MOTOR ACTIVITY EVALUATION IN Cr1:CD(TM)BR VAF/Plus(TM) RATS ADMINISTERED CHLORPROMAZINE  
AND D-AMPHETAMINE (POSITIVE CONTROL STUDY)

TABLE 1 (PAGE 1): MOTOR ACTIVITY - SUMMARY - FEMALE RATS

DOSAGE		V	VI	VII	VIII
D-AMPHETAMINE (MG/KG/DAY)		0(VEHICLE)	0.5	1.0	4.0
NUMBER OF RATS	N	15	15	15	15
NUMBER OF MOVEMENTS					
BLOCK 1	MEAN ± S.D.	75.1 ± 11.7	75.6 ± 11.0	73.6 ± 10.6	71.9 ± 11.4
BLOCK 2	MEAN ± S.D.	71.1 ± 8.8	74.9 ± 9.2	77.7 ± 8.9	66.8 ± 21.8
BLOCK 3	MEAN ± S.D.	70.1 ± 12.9	72.7 ± 12.7	74.4 ± 13.1	54.1 ± 29.7
BLOCK 4	MEAN ± S.D.	61.3 ± 19.0	71.3 ± 10.1	76.1 ± 13.8	46.5 ± 31.5
BLOCK 5	MEAN ± S.D.	54.6 ± 23.6	69.9 ± 18.6	74.9 ± 13.2	42.5 ± 32.9
BLOCK 6	MEAN ± S.D.	40.8 ± 27.4	69.7 ± 15.4	74.0 ± 10.5	40.9 ± 33.6
BLOCK 7	MEAN ± S.D.	28.1 ± 24.3	69.3 ± 14.0	74.9 ± 19.8	40.1 ± 30.8
BLOCK 8	MEAN ± S.D.	18.4 ± 23.2	66.0 ± 14.8	75.1 ± 20.2	42.2 ± 34.0
BLOCK 9	MEAN ± S.D.	15.3 ± 19.8	68.3 ± 17.4	71.4 ± 18.4	44.4 ± 39.0
BLOCK 10	MEAN ± S.D.	8.4 ± 18.8	69.3 ± 17.3	74.9 ± 18.5	38.9 ± 38.5
BLOCK 11	MEAN ± S.D.	3.7 ± 10.4	65.9 ± 11.7	71.3 ± 17.1	41.5 ± 43.7
BLOCK 12	MEAN ± S.D.	8.0 ± 16.0	68.5 ± 10.4	73.3 ± 12.5	43.8 ± 44.0
BLOCK 13	MEAN ± S.D.	4.4 ± 10.5	62.7 ± 16.7	79.1 ± 12.4	41.6 ± 40.6
BLOCK 14	MEAN ± S.D.	2.9 ± 5.7	66.9 ± 17.1	81.6 ± 15.6	43.7 ± 41.1
BLOCK 15	MEAN ± S.D.	9.1 ± 14.1	67.6 ± 22.4	77.4 ± 16.3	42.1 ± 39.0
BLOCK 16	MEAN ± S.D.	6.1 ± 13.2	64.8 ± 14.3	68.9 ± 13.8	45.2 ± 39.7
BLOCK 17	MEAN ± S.D.	3.2 ± 5.6	56.5 ± 16.7	68.3 ± 14.6	40.7 ± 37.9
BLOCK 18	MEAN ± S.D.	2.1 ± 2.2	48.3 ± 26.4	71.6 ± 15.6	43.5 ± 42.2
BLOCK 19	MEAN ± S.D.	1.6 ± 2.0	41.8 ± 26.3	70.7 ± 11.4	56.2 ± 39.7
BLOCK 20	MEAN ± S.D.	1.5 ± 2.2	43.1 ± 28.2	74.8 ± 13.6	49.9 ± 41.8
BLOCK 21	MEAN ± S.D.	2.4 ± 2.7	47.1 ± 27.0	65.5 ± 26.2	53.5 ± 41.0
BLOCK 22	MEAN ± S.D.	3.1 ± 6.2	49.4 ± 32.7	57.8 ± 29.2	50.6 ± 39.0
BLOCK 23	MEAN ± S.D.	8.1 ± 14.8	40.7 ± 31.6	56.5 ± 28.8	52.1 ± 38.9
BLOCK 24	MEAN ± S.D.	7.1 ± 16.6	37.4 ± 36.2	55.5 ± 29.4	57.5 ± 39.6
TOTAL	MEAN ± S.D.	506.4 ± 142.5	1467.5 ± 287.3**	1719.3 ± 262.4**	1150.2 ± 654.0**

STATISTICAL ANALYSES OF MOTOR ACTIVITY WERE RESTRICTED TO THE TOTAL NUMBER OF MOVEMENTS AND TOTAL TIME SPENT IN MOVEMENT.

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

\*\* Significantly different from the vehicle control group TOTAL ( $P \leq 0.01$ ).

PROTOCOL 012-016: MOTOR ACTIVITY EVALUATION IN Cr1:CD(TM)BR VAF/Plus(TM) RATS ADMINISTERED CHLORPROMAZINE AND D-AMPHETAMINE (POSITIVE CONTROL STUDY)

TABLE 1 (PAGE 2): MOTOR ACTIVITY - SUMMARY - FEMALE RATS

DOSAGE D-AMPHETAMINE (MG/KG/DAY)		V 0(VEHICLE)	VI 0.5	VII 1.0	VIII 4.0
NUMBER OF RATS	N	15	15	15	15
TIME (SECONDS) SPENT IN MOVEMENT					
BLOCK 1	MEAN ± S.D.	167.6 ± 28.0	190.6 ± 37.0	206.2 ± 30.6	173.4 ± 46.8
BLOCK 2	MEAN ± S.D.	141.8 ± 40.1	192.2 ± 36.1	197.4 ± 31.2	143.2 ± 83.1
BLOCK 3	MEAN ± S.D.	119.9 ± 34.2	185.7 ± 50.1	187.4 ± 45.2	107.7 ± 93.4
BLOCK 4	MEAN ± S.D.	101.7 ± 45.0	180.0 ± 46.8	174.9 ± 54.2	96.1 ± 99.7
BLOCK 5	MEAN ± S.D.	73.5 ± 39.4	151.5 ± 60.0	178.7 ± 42.1	98.6 ± 106.6
BLOCK 6	MEAN ± S.D.	49.1 ± 36.0	157.7 ± 61.8	181.1 ± 50.8	104.9 ± 113.0
BLOCK 7	MEAN ± S.D.	32.9 ± 40.5	159.5 ± 58.0	172.7 ± 57.9	108.9 ± 118.4
BLOCK 8	MEAN ± S.D.	25.9 ± 47.5	150.9 ± 64.6	178.7 ± 58.5	106.1 ± 108.9
BLOCK 9	MEAN ± S.D.	20.8 ± 46.1	142.3 ± 66.2	163.3 ± 70.4	98.0 ± 109.0
BLOCK 10	MEAN ± S.D.	13.4 ± 40.6	145.1 ± 68.2	163.7 ± 63.2	86.8 ± 108.0
BLOCK 11	MEAN ± S.D.	3.0 ± 10.0	129.7 ± 57.0	167.7 ± 62.5	91.1 ± 108.5
BLOCK 12	MEAN ± S.D.	14.8 ± 47.4	126.5 ± 57.2	156.5 ± 55.9	85.5 ± 106.5
BLOCK 13	MEAN ± S.D.	3.9 ± 12.6	128.6 ± 62.6	160.5 ± 50.2	94.5 ± 110.0
BLOCK 14	MEAN ± S.D.	1.9 ± 4.4	126.8 ± 54.7	165.1 ± 47.6	104.4 ± 113.5
BLOCK 15	MEAN ± S.D.	8.7 ± 17.3	115.5 ± 55.2	148.0 ± 53.4	98.6 ± 115.9
BLOCK 16	MEAN ± S.D.	7.0 ± 19.6	110.8 ± 49.2	149.5 ± 62.3	97.1 ± 110.6
BLOCK 17	MEAN ± S.D.	1.7 ± 4.4	90.2 ± 63.8	133.9 ± 56.6	98.4 ± 110.8
BLOCK 18	MEAN ± S.D.	0.5 ± 1.0	89.0 ± 80.8	145.1 ± 59.9	92.7 ± 105.1
BLOCK 19	MEAN ± S.D.	0.5 ± 1.0	78.5 ± 75.2	143.3 ± 62.8	104.4 ± 91.3
BLOCK 20	MEAN ± S.D.	0.4 ± 0.6	70.4 ± 64.5	143.9 ± 54.9	106.7 ± 108.0
BLOCK 21	MEAN ± S.D.	0.9 ± 1.8	78.3 ± 66.8	127.3 ± 71.4	96.4 ± 92.4
BLOCK 22	MEAN ± S.D.	2.1 ± 4.9	89.3 ± 74.8	108.7 ± 75.2	94.9 ± 86.7
BLOCK 23	MEAN ± S.D.	10.7 ± 30.3	68.7 ± 68.4	110.7 ± 86.4	82.1 ± 82.7
BLOCK 24	MEAN ± S.D.	10.6 ± 34.4	51.9 ± 55.2	110.4 ± 75.5	96.3 ± 85.9
TOTAL	MEAN ± S.D.	613.3 ± 323.6	3009.5 ± 1203.9**	3774.7 ± 1179.0**	2466.8 ± 2098.3**

STATISTICAL ANALYSES OF MOTOR ACTIVITY WERE RESTRICTED TO THE TOTAL NUMBER OF MOVEMENTS AND TOTAL TIME SPENT IN MOVEMENT.

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

\*\* Significantly different from the vehicle control group TOTAL ( $P \leq 0.01$ ).

PROTOCOL 012-016: MOTOR ACTIVITY EVALUATION IN Cr1:CD®BR VAF/Plus® RATS ADMINISTERED CHLORPROMAZINE  
AND D-AMPHETAMINE (POSITIVE CONTROL STUDY)

TABLE 3 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - MALE RATS

RATS TESTED a	15
APPEARED NORMAL	14
URINARY BLADDER: CONTAINED CLEAR FLUID AND WHITE STONE-LIKE MASSES, URINARY WALL THICK.	1b
KIDNEYS: LEFT; MODERATE DILATION, TAN AREA PRESENT THAT EXTENDED INTO THE PARENCHYMA, LARGE.	1b
RIGHT; MODERATE DILATION, TWO WHITE STONE-LIKE MASSES PRESENT, LARGE.	1b
URETERS: BOTH; SEVERE DILATION.	1b

- a. Refer to the individual clinical observations table (Table 4) for external observations confirmed at necropsy.  
b. Occurred in rat 19962.



PROTOCOL 012-016: MOTOR ACTIVITY EVALUATION IN Cr1:CD®BR VAF/Plus® RATS ADMINISTERED CHLORPROMAZINE  
AND D-AMPHETAMINE (POSITIVE CONTROL STUDY)

TABLE 5 (PAGE 1): NECROPSY OBSERVATIONS - INDIVIDUAL DATA - MALE RATS

RAT NUMBER	DAY OF NECROPSY	OBSERVATIONS <sup>a</sup>
19961	23	ALL TISSUES APPEARED NORMAL.
19962	23	URINARY BLADDER: CONTAINED APPROXIMATELY 2.8 mL OF CLEAR FLUID, NUMEROUS (<100) WHITE STONE-LIKE MASSES RANGING IN SIZE FROM (0.1 X 0.1 X 0.1 CM) TO (0.6 X 0.6 X 0.6 CM), URINARY WALL THICK. KIDNEYS: LEFT; PELVIS, MODERATE DILATION, TAN AREA PRESENT (1.0 CM IN DIAMETER) THAT EXTENDED INTO THE PARENCHYMA, LARGE. KIDNEYS: RIGHT; PELVIS, MODERATE DILATION, TWO WHITE STONE-LIKE MASSES PRESENT (0.3 X 0.3 X 0.2 CM, 0.4 X 0.4 X 0.5 CM), LARGE. URETERS: BOTH; SEVERE DILATION. ALL OTHER TISSUES APPEARED NORMAL.
19963	23	ALL TISSUES APPEARED NORMAL.
19964	23	ALL TISSUES APPEARED NORMAL.
19965	23	ALL TISSUES APPEARED NORMAL.
19966	23	ALL TISSUES APPEARED NORMAL.
19967	23	ALL TISSUES APPEARED NORMAL.
19968	23	ALL TISSUES APPEARED NORMAL.
19969	23	ALL TISSUES APPEARED NORMAL.
19970	23	ALL TISSUES APPEARED NORMAL.
19971	23	ALL TISSUES APPEARED NORMAL.
19972	23	ALL TISSUES APPEARED NORMAL.
19973	23	ALL TISSUES APPEARED NORMAL.
19974	23	ALL TISSUES APPEARED NORMAL.
19975	23	ALL TISSUES APPEARED NORMAL.

a. Refer to the individual clinical observations table (Table 4) for external observations confirmed at necropsy.

PROTOCOL 012-016: MOTOR ACTIVITY EVALUATION IN Crl:CD\*BR VAF/Plus® RATS ADMINISTERED CHLORPROMAZINE  
AND D-AMPHETAMINE (POSITIVE CONTROL STUDY)

TABLE 3 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - FEMALE RATS

RATS TESTED a	15
APPEARED NORMAL	15

a. Refer to the individual clinical observations table (Table 4) for external observations confirmed at necropsy.

PROTOCOL 012-016: MOTOR ACTIVITY EVALUATION IN Cr1:CD®BR VAF/Plus® RATS ADMINISTERED CHLORPROMAZINE  
AND D-AMPHETAMINE (POSITIVE CONTROL STUDY)

TABLE 5 (PAGE 1): NECROPSY OBSERVATIONS - INDIVIDUAL DATA - FEMALE RATS

RAT NUMBER	DAY OF NECROPSY	OBSERVATIONS <sup>a</sup>
4181	23	ALL TISSUES APPEARED NORMAL.
4182	23	ALL TISSUES APPEARED NORMAL.
4183	23	ALL TISSUES APPEARED NORMAL.
4184	23	ALL TISSUES APPEARED NORMAL.
4185	23	ALL TISSUES APPEARED NORMAL.
4186	23	ALL TISSUES APPEARED NORMAL.
4187	23	ALL TISSUES APPEARED NORMAL.
4188	23	ALL TISSUES APPEARED NORMAL.
4189	23	ALL TISSUES APPEARED NORMAL.
4190	23	ALL TISSUES APPEARED NORMAL.
4191	23	ALL TISSUES APPEARED NORMAL.
4192	23	ALL TISSUES APPEARED NORMAL.
4193	23	ALL TISSUES APPEARED NORMAL.
4194	23	ALL TISSUES APPEARED NORMAL.
4195	23	ALL TISSUES APPEARED NORMAL.

a. Refer to the individual clinical observations table (Table 4) for external observations confirmed at necropsy.

PROTOCOL 012-016: MOTOR ACTIVITY EVALUATION IN Crl:CD(TM)BR VAF/Plus(TM) RATS ADMINISTERED CHLORPROMAZINE  
AND D-AMPHETAMINE (POSITIVE CONTROL STUDY)

TABLE 5 (PAGE 1): MOTOR ACTIVITY - INDIVIDUAL DATA - MALE RATS

	DOSAGE I		0(VEHICLE)MG/KG/DAY		CHLORPROMAZINE							
RAT NUMBER	19961	19962	19963	19964	19965	19966	19967	19968	19969	19970	19971	19972
NUMBER OF MOVEMENTS												
BLOCK 1	73	58	72	70	75	65	79	51	91	77	76	54
BLOCK 2	77	76	75	71	72	97	71	58	100	77	78	53
BLOCK 3	78	74	77	37	58	83	55	60	82	59	62	48
BLOCK 4	42	78	57	75	67	66	54	57	78	58	43	51
BLOCK 5	83	39	70	86	77	40	48	65	79	90	4	31
BLOCK 6	60	75	40	68	69	39	31	43	80	78	2	44
BLOCK 7	16	84	19	71	80	0	25	43	39	79	9	25
BLOCK 8	45	50	4	71	72	1	1	17	40	64	3	33
BLOCK 9	35	76	0	0	60	0	0	8	28	71	2	63
BLOCK 10	4	50	0	0	70	0	0	1	4	66	13	55
BLOCK 11	2	40	0	1	57	1	0	2	1	75	2	34
BLOCK 12	4	0	0	0	73	0	0	0	47	82	3	45
BLOCK 13	0	0	0	3	51	0	0	2	60	44	5	10
BLOCK 14	0	0	0	0	74	3	2	0	17	62	5	0
BLOCK 15	1	0	0	7	52	0	0	6	22	0	2	0
BLOCK 16	5	2	0	10	62	0	1	2	22	1	39	0
BLOCK 17	2	0	4	4	69	0	1	4	15	0	9	8
BLOCK 18	1	0	0	0	67	0	4	5	0	10	0	15
BLOCK 19	1	0	4	10	19	0	0	4	1	4	0	3
BLOCK 20	31	2	0	8	2	0	0	3	1	18	3	5
BLOCK 21	0	2	0	0	0	0	0	2	38	1	1	0
BLOCK 22	11	0	0	2	0	0	2	3	46	6	4	8
BLOCK 23	19	6	0	0	0	1	0	0	87	1	5	4
BLOCK 24	0	0	5	3	0	0	0	1	4	5	4	0
TOTAL	590	712	427	597	1226	396	374	437	982	1028	374	589

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

PROTOCOL 012-016: MOTOR ACTIVITY EVALUATION IN Cr1:CD(TM)BR VAF/Plus(TM) RATS ADMINISTERED CHLORPROMAZINE  
AND D-AMPHETAMINE (POSITIVE CONTROL STUDY)

TABLE 5 (PAGE 2): MOTOR ACTIVITY - INDIVIDUAL DATA - MALE RATS

RAT NUMBER	DOSAGE I	O(VEHICLE)MG/KG/DAY		CHLORPROMAZINE
	19973	19974	19975	
NUMBER OF MOVEMENTS				
BLOCK 1	65	82	81	
BLOCK 2	52	84	86	
BLOCK 3	44	81	76	
BLOCK 4	46	83	70	
BLOCK 5	57	81	45	
BLOCK 6	55	69	52	
BLOCK 7	26	14	31	
BLOCK 8	46	13	24	
BLOCK 9	17	7	9	
BLOCK 10	0	0	8	
BLOCK 11	0	13	0	
BLOCK 12	0	56	0	
BLOCK 13	1	80	0	
BLOCK 14	3	71	0	
BLOCK 15	0	23	0	
BLOCK 16	0	0	2	
BLOCK 17	3	4	0	
BLOCK 18	0	4	0	
BLOCK 19	0	1	0	
BLOCK 20	1	2	0	
BLOCK 21	0	6	0	
BLOCK 22	4	0	0	
BLOCK 23	0	0	1	
BLOCK 24	0	0	1	
TOTAL	420	774	486	

TOTAL - SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

PROTOCOL 012-016: MOTOR ACTIVITY EVALUATION IN Cr1:CD(TM)BR VAF/Plus(TM) RATS ADMINISTERED CHLORPROMAZINE AND D-AMPHETAMINE (POSITIVE CONTROL STUDY)

TABLE 5 (PAGE 3): MOTOR ACTIVITY - INDIVIDUAL DATA - MALE RATS

RAT NUMBER	DOSAGE I		O(VEHICLE)MG/KG/DAY		CHLORPROMAZINE							
	19961	19962	19963	19964	19965	19966	19967	19968	19969	19970	19971	19972
TIME (SECONDS) SPENT IN MOVEMENT												
BLOCK 1	132	117	189	183	205	85	122	155	187	187	167	231
BLOCK 2	153	177	164	181	188	131	109	117	138	197	153	156
BLOCK 3	118	122	164	98	181	129	80	96	177	193	85	98
BLOCK 4	55	117	194	96	189	69	64	108	110	77	61	141
BLOCK 5	128	41	109	98	168	42	57	173	113	112	1	50
BLOCK 6	80	102	71	126	161	34	36	78	84	168	0	60
BLOCK 7	25	95	27	128	103	0	33	95	45	136	3	45
BLOCK 8	70	64	1	52	147	0	0	17	44	98	0	46
BLOCK 9	30	131	0	0	103	0	0	5	42	110	0	149
BLOCK 10	2	52	0	0	214	0	0	0	0	175	10	126
BLOCK 11	1	29	0	0	77	0	0	1	0	88	0	56
BLOCK 12	3	0	0	0	138	0	0	0	66	133	2	91
BLOCK 13	0	0	0	1	59	0	0	0	115	60	4	14
BLOCK 14	0	0	0	0	98	2	0	0	20	53	1	0
BLOCK 15	0	0	0	4	70	0	0	4	20	0	0	0
BLOCK 16	4	1	0	4	97	0	0	1	16	0	43	0
BLOCK 17	1	0	2	2	110	0	0	1	15	0	5	12
BLOCK 18	0	0	0	0	52	0	0	3	0	5	0	27
BLOCK 19	0	0	1	8	29	0	0	7	0	0	0	1
BLOCK 20	33	0	0	5	0	0	0	2	0	11	2	2
BLOCK 21	0	0	0	0	0	0	0	0	48	0	0	0
BLOCK 22	9	0	0	0	0	0	1	0	69	7	3	7
BLOCK 23	13	3	0	0	0	0	0	0	96	0	3	2
BLOCK 24	0	0	1	3	0	0	0	0	2	2	2	0
TOTAL	857	1051	923	989	2389	492	502	863	1407	1812	545	1314

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

PROTOCOL 012-016: MOTOR ACTIVITY EVALUATION IN Crl:CD(TM)BR VAF/Plus(TM) RATS ADMINISTERED CHLORPROMAZINE AND D-AMPHETAMINE (POSITIVE CONTROL STUDY)

TABLE 5 (PAGE 4): MOTOR ACTIVITY - INDIVIDUAL DATA - MALE RATS

RAT NUMBER	DOSAGE I	O(VEHICLE)MG/KG/DAY		CHLORPROMAZINE
	19973	19974	19975	
TIME (SECONDS) SPENT IN MOVEMENT				
BLOCK 1	129	178	181	
BLOCK 2	70	170	142	
BLOCK 3	72	145	122	
BLOCK 4	94	200	160	
BLOCK 5	125	119	76	
BLOCK 6	61	66	103	
BLOCK 7	28	8	26	
BLOCK 8	40	3	13	
BLOCK 9	12	7	8	
BLOCK 10	0	0	5	
BLOCK 11	0	5	0	
BLOCK 12	0	107	0	
BLOCK 13	0	169	0	
BLOCK 14	2	62	0	
BLOCK 15	0	16	0	
BLOCK 16	0	0	0	
BLOCK 17	4	0	0	
BLOCK 18	0	2	0	
BLOCK 19	0	0	0	
BLOCK 20	0	0	0	
BLOCK 21	0	3	0	
BLOCK 22	4	0	0	
BLOCK 23	0	0	0	
BLOCK 24	0	0	1	
TOTAL	641	1260	837	

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

PROTOCOL 012-016: MOTOR ACTIVITY EVALUATION IN Crl:CD(TM)BR VAF/Plus(TM) RATS ADMINISTERED CHLORPROMAZINE AND D-AMPHETAMINE (POSITIVE CONTROL STUDY)

TABLE 5 (PAGE 1): MOTOR ACTIVITY - INDIVIDUAL DATA - FEMALE RATS

	DOSAGE I		0(VEHICLE)MG/KG/DAY		CHLORPROMAZINE							
RAT NUMBER	4181	4182	4183	4184	4185	4186	4187	4188	4189	4190	4191	4192
NUMBER OF MOVEMENTS												
BLOCK 1	79	75	79	70	90	74	58	56	64	83	80	75
BLOCK 2	95	82	72	65	77	88	77	74	73	78	80	85
BLOCK 3	73	79	61	58	88	69	86	62	70	73	70	90
BLOCK 4	75	73	58	72	82	73	64	67	73	86	64	49
BLOCK 5	56	65	51	73	82	59	61	70	51	70	58	29
BLOCK 6	2	73	54	62	78	67	22	81	46	60	56	0
BLOCK 7	3	73	8	64	42	30	7	49	34	22	18	2
BLOCK 8	1	67	3	68	27	69	0	64	51	57	4	0
BLOCK 9	5	68	0	57	0	34	0	11	55	43	6	1
BLOCK 10	0	54	0	14	0	0	1	3	24	10	10	18
BLOCK 11	7	47	2	0	1	6	0	5	30	0	1	50
BLOCK 12	0	20	52	0	0	0	6	1	35	0	1	37
BLOCK 13	4	43	19	1	5	0	0	2	59	4	0	8
BLOCK 14	0	47	0	2	1	1	0	0	57	0	0	0
BLOCK 15	0	63	8	12	1	14	0	5	68	3	3	1
BLOCK 16	51	45	1	3	2	54	1	3	60	7	0	1
BLOCK 17	16	31	1	4	1	36	0	0	59	0	12	11
BLOCK 18	3	43	26	28	0	3	0	3	27	0	4	12
BLOCK 19	2	9	1	0	0	0	0	3	0	2	57	7
BLOCK 20	1	8	0	0	8	3	0	0	7	1	59	4
BLOCK 21	1	0	1	2	0	2	0	0	0	0	77	0
BLOCK 22	2	0	1	6	0	0	0	0	3	1	59	0
BLOCK 23	1	1	1	2	1	0	0	3	0	0	30	9
BLOCK 24	2	1	2	5	3	4	0	0	0	0	0	1
TOTAL	479	1067	501	668	589	686	383	562	946	600	749	491

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.



PROTOCOL 012-016: MOTOR ACTIVITY EVALUATION IN Crl:CD(TM)BR VAF/Plus(TM) RATS ADMINISTERED CHLORPROMAZINE AND D-AMPHETAMINE (POSITIVE CONTROL STUDY)

TABLE 5 (PAGE 2): MOTOR ACTIVITY - INDIVIDUAL DATA - FEMALE RATS

	DOSAGE I			O(VEHICLE)MG/KG/DAY			CHLORPROMAZINE		
RAT NUMBER	4193	4194	4195						
NUMBER OF MOVEMENTS									
BLOCK 1	77	78	70						
BLOCK 2	69	80	70						
BLOCK 3	51	64	61						
BLOCK 4	60	95	63						
BLOCK 5	33	51	32						
BLOCK 6	1	4	8						
BLOCK 7	0	5	1						
BLOCK 8	4	3	5						
BLOCK 9	0	0	2						
BLOCK 10	6	1	9						
BLOCK 11	0	8	5						
BLOCK 12	8	7	0						
BLOCK 13	0	3	2						
BLOCK 14	0	21	0						
BLOCK 15	1	0	5						
BLOCK 16	0	11	2						
BLOCK 17	0	12	0						
BLOCK 18	56	3	27						
BLOCK 19	5	1	41						
BLOCK 20	0	3	18						
BLOCK 21	1	6	0						
BLOCK 22	0	1	5						
BLOCK 23	0	3	3						
BLOCK 24	0	1	0						
TOTAL	372	461	429						

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

PROTOCOL 012-016: MOTOR ACTIVITY EVALUATION IN Cr1:CD(TM)BR VAF/Plus(TM) RATS ADMINISTERED CHLORPROMAZINE AND D-AMPHETAMINE (POSITIVE CONTROL STUDY)

TABLE 5 (PAGE 3): MOTOR ACTIVITY - INDIVIDUAL DATA - FEMALE RATS

DOSAGE I		0(VEHICLE)MG/KG/DAY				CHLORPROMAZINE						
RAT NUMBER	4181	4182	4183	4184	4185	4186	4187	4188	4189	4190	4191	4192
TIME (SECONDS) SPENT IN MOVEMENT												
BLOCK 1	106	166	171	203	144	120	129	238	128	194	165	127
BLOCK 2	115	149	154	144	141	144	156	173	166	180	169	111
BLOCK 3	112	113	101	191	169	103	113	161	124	162	135	129
BLOCK 4	76	95	89	159	143	112	94	153	110	122	114	48
BLOCK 5	59	112	75	153	95	51	77	162	96	95	129	24
BLOCK 6	3	113	78	170	139	108	12	123	72	60	95	0
BLOCK 7	0	117	9	122	50	28	2	101	18	16	14	0
BLOCK 8	0	63	0	140	25	123	0	93	62	71	2	0
BLOCK 9	2	81	0	79	0	37	0	6	78	63	1	0
BLOCK 10	0	89	0	8	0	0	0	1	16	3	5	10
BLOCK 11	2	45	0	0	0	3	0	4	25	0	0	31
BLOCK 12	0	10	72	0	0	0	2	1	43	0	0	35
BLOCK 13	1	46	18	0	1	0	0	0	63	1	0	1
BLOCK 14	0	54	0	2	0	0	0	0	64	0	0	0
BLOCK 15	0	86	3	8	0	7	0	2	120	0	2	0
BLOCK 16	49	54	0	1	0	78	0	0	107	3	0	0
BLOCK 17	20	10	0	1	0	25	0	0	53	0	7	5
BLOCK 18	1	45	25	23	0	2	0	2	24	0	0	6
BLOCK 19	0	6	0	0	0	0	0	0	0	0	62	1
BLOCK 20	0	2	0	0	3	1	0	0	4	0	63	1
BLOCK 21	0	0	0	0	0	0	0	0	0	0	131	0
BLOCK 22	1	0	0	4	0	0	0	0	0	0	63	0
BLOCK 23	0	0	0	0	0	0	0	1	0	0	28	4
BLOCK 24	0	0	0	2	1	4	0	0	0	0	0	0
TOTAL	547	1456	795	1410	911	946	585	1221	1373	970	1185	533

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

PROTOCOL 012-016: MOTOR ACTIVITY EVALUATION IN Cr1:CD(TM)BR VAF/Plus(TM) RATS ADMINISTERED CHLORPROMAZINE  
AND D-AMPHETAMINE (POSITIVE CONTROL STUDY)

TABLE 5 (PAGE 4): MOTOR ACTIVITY - INDIVIDUAL DATA - FEMALE RATS

	DOSAGE I	O(VEHICLE)MG/KG/DAY	CHLORPROMAZINE
RAT NUMBER	4193	4194	4195
TIME (SECONDS) SPENT IN MOVEMENT			
BLOCK 1	163	116	174
BLOCK 2	88	116	168
BLOCK 3	39	105	126
BLOCK 4	109	99	93
BLOCK 5	26	54	22
BLOCK 6	0	2	1
BLOCK 7	0	4	0
BLOCK 8	1	1	1
BLOCK 9	0	0	0
BLOCK 10	2	0	7
BLOCK 11	0	5	2
BLOCK 12	3	9	0
BLOCK 13	0	0	0
BLOCK 14	0	14	0
BLOCK 15	0	0	5
BLOCK 16	0	6	2
BLOCK 17	0	6	0
BLOCK 18	91	0	38
BLOCK 19	3	0	57
BLOCK 20	0	4	13
BLOCK 21	0	1	0
BLOCK 22	0	0	2
BLOCK 23	0	2	3
BLOCK 24	0	0	0
TOTAL	525	544	714

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

ROTOCOL 012-016: MOTOR ACTIVITY EVALUATION IN Crl:CD(TM)BR VAF/Plus(TM) RATS ADMINISTERED CHLORPROMAZINE  
AND D-AMPHETAMINE (POSITIVE CONTROL STUDY)

TABLE 5 (PAGE 1): MOTOR ACTIVITY - INDIVIDUAL DATA - MALE RATS

	DOSAGE V	O(VEHICLE)MG/KG/DAY				D-AMPHETAMINE						
RAT NUMBER	19961	19962	19963	19964	19965	19966	19967	19968	19969	19970	19971	19972
NUMBER OF MOVEMENTS												
BLOCK 1	84	79	71	74	81	90	71	71	68	82	77	81
BLOCK 2	67	68	81	62	69	78	80	68	60	96	85	69
BLOCK 3	85	84	77	72	69	70	89	78	69	66	64	64
BLOCK 4	60	63	13	76	68	57	84	62	58	64	50	83
BLOCK 5	42	74	41	73	77	90	54	58	25	79	54	77
BLOCK 6	54	58	0	55	65	64	46	63	0	66	67	62
BLOCK 7	27	71	0	54	84	0	54	64	0	79	25	61
BLOCK 8	62	82	2	21	70	1	17	56	0	81	3	17
BLOCK 9	13	27	0	3	53	0	71	59	0	64	0	0
BLOCK 10	75	11	5	1	49	0	56	64	0	29	0	2
BLOCK 11	3	13	28	1	68	0	25	65	1	77	4	13
BLOCK 12	7	0	68	1	42	3	45	64	1	28	3	0
BLOCK 13	0	1	69	3	19	3	27	32	2	2	16	4
BLOCK 14	8	0	36	0	0	0	0	33	0	0	0	1
BLOCK 15	1	0	11	0	0	0	3	38	3	0	0	2
BLOCK 16	5	2	12	5	1	3	0	42	7	3	0	0
BLOCK 17	3	0	33	8	1	0	0	8	1	7	3	2
BLOCK 18	0	0	40	1	2	0	7	2	0	2	7	6
BLOCK 19	11	4	7	2	0	0	1	1	3	3	47	2
BLOCK 20	0	0	6	0	3	8	0	3	0	16	4	4
BLOCK 21	0	3	31	0	0	2	0	1	0	1	4	0
BLOCK 22	0	5	0	0	1	0	1	4	13	2	9	26
BLOCK 23	2	0	0	0	5	3	7	0	3	2	2	7
BLOCK 24	6	0	12	0	1	2	3	0	0	0	11	2
TOTAL	615	645	643	512	828	474	741	936	314	849	535	585

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

PROTOCOL 012-016: MOTOR ACTIVITY EVALUATION IN Cr1:CD(TM)BR VAF/Plus(TM) RATS ADMINISTERED CHLORPROMAZINE  
AND D-AMPHETAMINE (POSITIVE CONTROL STUDY)

TABLE 5 (PAGE 2): MOTOR ACTIVITY - INDIVIDUAL DATA - MALE RATS

RAT NUMBER	DOSAGE V	O(VEHICLE)MG/KG/DAY		D-AMPHETAMINE
	19973	19974	19975	
NUMBER OF MOVEMENTS				
BLOCK 1	69	77	91	
BLOCK 2	71	84	71	
BLOCK 3	57	79	80	
BLOCK 4	60	83	79	
BLOCK 5	45	73	61	
BLOCK 6	0	59	76	
BLOCK 7	0	35	70	
BLOCK 8	0	21	75	
BLOCK 9	14	0	71	
BLOCK 10	0	1	74	
BLOCK 11	0	5	41	
BLOCK 12	0	5	21	
BLOCK 13	0	0	17	
BLOCK 14	0	0	6	
BLOCK 15	1	2	1	
BLOCK 16	0	8	5	
BLOCK 17	0	1	0	
BLOCK 18	1	2	0	
BLOCK 19	22	5	0	
BLOCK 20	1	2	1	
BLOCK 21	19	4	0	
BLOCK 22	0	0	0	
BLOCK 23	0	0	4	
BLOCK 24	3	0	1	
TOTAL	363	546	845	

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

PROTOCOL 012-016: MOTOR ACTIVITY EVALUATION IN Cr1:CD(TM)BR VAF/Plus(TM) RATS ADMINISTERED CHLORPROMAZINE AND D-AMPHETAMINE (POSITIVE CONTROL STUDY)

TABLE 5 (PAGE 3): MOTOR ACTIVITY - INDIVIDUAL DATA - MALE RATS

	DOSAGE V		O(VEHICLE)MG/KG/DAY		D-AMPHETAMINE							
RAT NUMBER	19961	19962	19963	19964	19965	19966	19967	19968	19969	19970	19971	19972
TIME (SECONDS) SPENT IN MOVEMENT												
BLOCK 1	149	130	160	197	196	143	87	188	199	151	196	185
BLOCK 2	108	138	154	181	211	143	90	162	125	127	165	126
BLOCK 3	108	188	107	125	187	87	127	162	139	143	87	82
BLOCK 4	66	117	9	157	181	54	88	114	105	145	64	106
BLOCK 5	53	116	62	92	202	102	73	137	17	135	74	116
BLOCK 6	56	80	0	87	152	86	45	109	0	154	93	91
BLOCK 7	27	108	0	82	146	0	77	98	0	112	22	89
BLOCK 8	115	120	2	20	91	0	13	67	0	170	0	14
BLOCK 9	11	21	0	0	122	0	91	82	0	85	0	0
BLOCK 10	142	8	4	0	216	0	74	134	0	34	0	0
BLOCK 11	0	9	29	0	101	0	15	160	0	99	2	11
BLOCK 12	8	0	96	0	59	0	43	99	0	33	2	0
BLOCK 13	0	0	105	3	15	2	37	33	0	1	18	1
BLOCK 14	6	0	56	0	0	0	0	29	0	0	0	0
BLOCK 15	0	0	9	0	0	0	0	56	1	0	0	1
BLOCK 16	3	0	7	4	0	2	0	58	4	0	0	0
BLOCK 17	0	0	58	9	0	0	0	6	1	4	3	0
BLOCK 18	0	0	50	0	1	0	4	2	0	1	3	4
BLOCK 19	11	2	3	0	0	0	0	0	1	0	85	0
BLOCK 20	0	0	7	0	2	5	0	0	0	14	2	3
BLOCK 21	0	1	38	0	0	1	0	1	0	1	0	0
BLOCK 22	0	5	0	0	0	0	0	0	10	1	6	25
BLOCK 23	0	0	0	0	1	1	2	0	1	1	1	7
BLOCK 24	4	0	9	0	0	3	1	0	0	0	12	2
TOTAL	867	1043	965	957	1883	629	867	1697	603	1411	835	863

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

PROTOCOL 012-016: MOTOR ACTIVITY EVALUATION IN Cr1:CD(TM)BR VAF/Plus(TM) RATS ADMINISTERED CHLORPROMAZINE  
AND D-AMPHETAMINE (POSITIVE CONTROL STUDY)

TABLE 5 (PAGE 4): MOTOR ACTIVITY - INDIVIDUAL DATA - MALE RATS

RAT NUMBER	DOSAGE V	0(VEHICLE)MG/KG/DAY		D-AMPHETAMINE
	19973	19974	19975	
TIME (SECONDS) SPENT IN MOVEMENT				
BLOCK 1	127	235	166	
BLOCK 2	150	154	149	
BLOCK 3	90	146	142	
BLOCK 4	127	134	151	
BLOCK 5	53	161	129	
BLOCK 6	0	70	166	
BLOCK 7	0	42	137	
BLOCK 8	0	17	96	
BLOCK 9	14	0	103	
BLOCK 10	0	0	145	
BLOCK 11	0	3	56	
BLOCK 12	0	1	24	
BLOCK 13	0	0	9	
BLOCK 14	0	0	2	
BLOCK 15	0	1	0	
BLOCK 16	0	4	3	
BLOCK 17	0	0	0	
BLOCK 18	0	0	0	
BLOCK 19	12	2	0	
BLOCK 20	0	1	0	
BLOCK 21	25	2	0	
BLOCK 22	0	0	0	
BLOCK 23	0	0	3	
BLOCK 24	2	0	0	
TOTAL	600	973	1481	

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

PROTOCOL 012-016: MOTOR ACTIVITY EVALUATION IN Crl:CD(TM)BR VAF/Plus(TM) RATS ADMINISTERED CHLORPROMAZINE AND D-AMPHETAMINE (POSITIVE CONTROL STUDY)

TABLE 5 (PAGE 1): MOTOR ACTIVITY - INDIVIDUAL DATA - FEMALE RATS

	DOSAGE V		O(VEHICLE)MG/KG/DAY		D-AMPHETAMINE							
RAT NUMBER	4181	4182	4183	4184	4185	4186	4187	4188	4189	4190	4191	4192
NUMBER OF MOVEMENTS												
BLOCK 1	68	80	98	71	69	86	72	47	70	66	85	72
BLOCK 2	69	62	73	63	79	75	77	53	63	70	73	65
BLOCK 3	70	67	63	75	86	73	49	79	80	74	77	36
BLOCK 4	61	56	78	74	71	87	63	60	69	62	20	21
BLOCK 5	68	10	75	71	74	41	51	51	45	65	31	9
BLOCK 6	72	50	23	57	75	18	1	0	58	19	5	41
BLOCK 7	40	6	2	65	46	14	25	0	34	0	0	47
BLOCK 8	67	0	0	58	2	0	4	14	8	0	0	21
BLOCK 9	39	0	17	62	1	1	0	0	15	1	0	0
BLOCK 10	0	0	1	72	4	3	1	6	0	0	0	1
BLOCK 11	1	2	0	41	1	1	0	3	0	0	0	0
BLOCK 12	19	3	1	58	5	7	0	0	0	0	0	27
BLOCK 13	1	14	0	40	0	1	0	5	0	0	1	2
BLOCK 14	6	1	4	0	1	0	0	1	3	0	0	22
BLOCK 15	24	0	48	2	0	1	4	7	0	3	11	30
BLOCK 16	52	0	8	0	10	2	1	0	4	0	1	6
BLOCK 17	22	3	0	3	1	0	0	6	4	5	0	0
BLOCK 18	1	1	4	1	1	1	0	1	4	1	1	8
BLOCK 19	7	0	1	2	0	2	0	0	0	4	0	4
BLOCK 20	0	0	3	0	0	5	0	3	0	1	0	0
BLOCK 21	0	4	7	0	0	1	1	0	0	7	7	2
BLOCK 22	1	1	1	0	2	1	0	11	2	3	0	23
BLOCK 23	26	9	2	1	0	1	0	54	0	18	0	5
BLOCK 24	16	0	2	0	0	9	0	65	0	4	0	1
TOTAL	730	369	511	816	528	430	349	466	459	403	312	443

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.



PROTOCOL 012-016: MOTOR ACTIVITY EVALUATION IN Crl:CD(TM)BR VAF/Plus(TM) RATS ADMINISTERED CHLORPROMAZINE AND D-AMPHETAMINE (POSITIVE CONTROL STUDY)

TABLE 5 (PAGE 2): MOTOR ACTIVITY - INDIVIDUAL DATA - FEMALE RATS

	DOSAGE V	0(VEHICLE)MG/KG/DAY	D-AMPHETAMINE
RAT NUMBER	4193	4194	4195
NUMBER OF MOVEMENTS			
BLOCK 1	78	79	85
BLOCK 2	83	86	76
BLOCK 3	76	80	67
BLOCK 4	80	65	52
BLOCK 5	84	77	67
BLOCK 6	73	67	53
BLOCK 7	59	64	19
BLOCK 8	24	53	25
BLOCK 9	23	30	41
BLOCK 10	25	10	3
BLOCK 11	0	1	5
BLOCK 12	0	0	0
BLOCK 13	2	0	0
BLOCK 14	6	0	0
BLOCK 15	0	7	0
BLOCK 16	0	7	0
BLOCK 17	2	0	2
BLOCK 18	2	1	5
BLOCK 19	1	2	1
BLOCK 20	7	0	3
BLOCK 21	2	1	4
BLOCK 22	1	0	0
BLOCK 23	1	2	2
BLOCK 24	1	4	4
TOTAL	630	636	514

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

PROTOCOL 012-016: MOTOR ACTIVITY EVALUATION IN Crl:CD(TM)BR VAF/Plus(TM) RATS ADMINISTERED CHLORPROMAZINE AND D-AMPHETAMINE (POSITIVE CONTROL STUDY)

TABLE 5 (PAGE 3): MOTOR ACTIVITY - INDIVIDUAL DATA - FEMALE RATS

RAT NUMBER	DOSAGE V		O(VEHICLE)MG/KG/DAY		D-AMPHETAMINE							
	4181	4182	4183	4184	4185	4186	4187	4188	4189	4190	4191	4192
TIME (SECONDS) SPENT IN MOVEMENT												
BLOCK 1	137	166	157	165	189	179	132	234	191	204	146	148
BLOCK 2	101	137	95	168	154	110	108	220	191	180	127	73
BLOCK 3	90	118	95	154	139	116	74	148	165	110	125	46
BLOCK 4	76	65	126	145	154	115	90	146	121	129	23	16
BLOCK 5	99	4	80	150	104	48	80	69	49	61	47	7
BLOCK 6	72	75	22	81	83	8	0	0	62	38	3	46
BLOCK 7	28	3	1	125	38	7	15	0	35	0	0	51
BLOCK 8	111	0	0	159	2	0	2	6	4	0	0	15
BLOCK 9	31	0	8	181	1	0	0	0	14	0	0	0
BLOCK 10	0	0	0	158	1	2	0	7	0	0	0	0
BLOCK 11	0	0	0	39	0	0	0	2	0	0	0	0
BLOCK 12	10	1	0	185	2	3	0	0	0	0	0	21
BLOCK 13	0	7	0	49	0	0	0	3	0	0	0	0
BLOCK 14	3	0	4	0	0	0	0	0	1	0	0	17
BLOCK 15	18	0	62	0	0	0	2	4	0	2	5	33
BLOCK 16	77	0	8	0	8	0	0	0	1	0	0	5
BLOCK 17	17	0	0	1	0	0	0	3	1	3	0	0
BLOCK 18	0	0	2	0	0	0	0	1	0	0	0	3
BLOCK 19	2	0	0	0	0	0	0	0	0	1	0	2
BLOCK 20	0	0	1	0	0	1	0	2	0	0	0	0
BLOCK 21	0	0	5	0	0	0	0	0	0	5	3	0
BLOCK 22	1	0	0	0	1	0	0	10	0	2	0	17
BLOCK 23	18	2	1	0	0	0	0	118	0	19	0	0
BLOCK 24	15	0	0	0	0	4	0	134	0	2	0	0
TOTAL	906	578	667	1760	876	593	503	1107	835	756	479	500

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

PROTOCOL 012-016: MOTOR ACTIVITY EVALUATION IN Cr1:CD(TM)BR VAF/Plus(TM) RATS ADMINISTERED CHLORPROMAZINE  
AND D-AMPHETAMINE (POSITIVE CONTROL STUDY)

TABLE 5 (PAGE 4): MOTOR ACTIVITY - INDIVIDUAL DATA - FEMALE RATS

	DOSAGE V	O(VEHICLE)MG/KG/DAY	D-AMPHETAMINE
RAT NUMBER	4193	4194	4195
TIME (SECONDS) SPENT IN MOVEMENT			
BLOCK 1	156	139	171
BLOCK 2	164	157	142
BLOCK 3	146	108	164
BLOCK 4	158	90	71
BLOCK 5	119	96	90
BLOCK 6	111	85	51
BLOCK 7	71	110	9
BLOCK 8	11	56	22
BLOCK 9	19	18	40
BLOCK 10	28	2	3
BLOCK 11	0	0	4
BLOCK 12	0	0	0
BLOCK 13	0	0	0
BLOCK 14	4	0	0
BLOCK 15	0	4	0
BLOCK 16	0	6	0
BLOCK 17	0	0	0
BLOCK 18	0	0	2
BLOCK 19	0	3	0
BLOCK 20	1	0	1
BLOCK 21	0	0	1
BLOCK 22	0	0	0
BLOCK 23	0	0	3
BLOCK 24	0	2	2
TOTAL	988	876	776

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

APPENDIX 7  
MOTOR ACTIVITY POSITIVE CONTROL #3

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN  
Crl:CD® VAF/Plus® RATS

A. Methodology

1. General Experimental Design and Procedures

The purpose of this study was to evaluate positive control substances using a functional observational battery (FOB) and motor activity test. Data obtained in this study are intended for use as positive control information for validation of the Test Facility's functional observational battery and motor activity test, and for certification of technicians conducting the testing, in accordance with the testing guidelines of the U.S. Environmental Protection Agency (EPA). The study was conducted in compliance with EPA Good Laboratory Practices (GLPs).

The test substances (acrylamide, carbaryl, DDT, IDPN and triadimefon) and their vehicles, were administered to male and female rats for one to nine days. There were four rats per sex randomly assigned to each of the seven dosage groups.

<u>Dosage Group</u>	<u>Dosage (mg/kg/day)</u>	<u>Concentration (mg/mL)</u>	<u>Volume (mL/kg/day)</u>
I	0 (Vehicle, 0.9% saline)	0	1
II	0 (Vehicle, corn oil)	0	5
III	40 (acrylamide)	40	1
IV	200 (IDPN)	200	1
V	75 (carbaryl)	15	5
VI	75 (DDT)	15	5
VII	200 (triadimefon)	40	5

The study was conducted in two replicates. One-half of the rats per sex in each dosage group were assigned to Replicate 1. The remaining rats in each dosage group were assigned to Replicate 2. Testing procedures in Replicate 1 preceded those in Replicate 2 by one to three days. In each replicate, the vehicles [sterile saline (0.9%) (used for acrylamide and IDPN) and corn oil (used for carbaryl, DDT and triadimefon)] and solutions/suspensions of the test substances in the vehicle were administered to the rats. The dosage volume was 1 mL/kg for the acrylamide and IDPN solutions and their vehicle. The dosage volume for solution/suspensions of DDT, carbaryl and triadimefon was 5 mL/kg.

In addition to observations for viability, general health, and signs of pharmacologic or toxicologic effects, specific evaluations of autonomic function, reactivity and sensitivity, excitability, grip strength, gait and sensorimotor coordination were made as part of a functional observational battery (FOB). Motor activity was also measured on the same day as the FOB during 1.5 hours test sessions using an automated apparatus. These tests were conducted before and during the dosage period. Body weights were recorded twice during the acclimation period, on the day of predosage FOB and motor activity tests, and daily during the dosage and postdosage periods. Feed consumption values were measured on the day of predosage FOB and motor activity tests, and daily during the dosage and postdosage periods<sup>a</sup>.

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a. Feed consumption values were not recorded for rats in Groups I, III and IV after day 13 of observation because the diet was changed from the meal form to the pellet form and accurate measurements could not be made on pellet consumption.

After the completion of the functional observational battery and motor activity test, the rats administered acrylamide, IDPN and their vehicle were perfused in situ with neutral buffered 10% formalin. After necropsy, the head, vertebral column and hindlimbs of these rats were removed and dissected to expose the brain, spinal cord and peripheral nerves in the hindlimbs and then immersed in the fixative for possible future evaluation. Rats in the other dosage groups were sacrificed by carbon dioxide asphyxiation and necropsied. Gross lesions were fixed in neutral buffered 10% formalin for possible future evaluation. The carcasses were discarded.

## 2. Conduct of Study

The in-life phases of the study occurred as follows:

<u>Phase</u>	<u>Dates</u>
<u>Replicate 1</u>	
Acclimation Period	09/09/91 - 09/17/91
Predosage Period FOB and Motor Activity:	
Dosage Groups I, III, IV	09/18/91
Dosage Groups II, V, VI, VII	09/20/91
Dosage Period:	
Dosage Groups I, III	09/18/91 - 09/26/91
Dosage Group IV	09/18/91 - 09/20/91
Dosage Groups II, V, VI, VII	09/26/91
Dosage Period FOB and Motor Activity:	
Dosage Groups I, III, IV	09/24/91
Dosage Groups II, V, VI, VII	09/26/91

<u>Phase</u>	<u>Dates</u>
<u>Replicate 1 (cont'd)</u>	
Postdosage Period FOB and Motor Activity: Dosage Groups I, III, IV	09/30/91
Scheduled Sacrifice: Dosage Groups I - VII	10/02/91 - 10/11/91
<u>Replicate 2</u>	
Acclimation Period	09/09/91 - 09/17/91
Predosage Period FOB and Motor Activity: Dosage Groups I, III, IV Dosage Groups II, V, VI, VII	09/19/91 09/23/91
Dosage Period: Dosage Groups I, III Dosage Group IV Dosage Groups II, V, VI, VII	09/19/91 - 09/27/91 09/19/91 - 09/21/91 09/27/91
Dosage Period FOB and Motor Activity: Dosage Groups I, III, IV Dosage Groups II, V, VI, VII	09/25/91 09/27/91
Postdosage Period FOB and Motor Activity: Dosage Groups I, III, IV Dosage Groups II, VI	10/01/91 10/10/91
Scheduled Sacrifice: Dosage Groups I - VII	10/02/91 - 10/11/91

This study was sponsored by Argus Research Laboratories, Inc., 905 Sheehy Drive, Horsham, Pennsylvania 19044. The Sponsor's Representative was Alan M. Hoberman, Ph.D., D.A.B.T. (Director of Research).



The study was conducted by and at Argus Research Laboratories, Inc., 905 Sheehy Drive, Horsham, Pennsylvania 19044. The Study Director was John A. Foss, Ph.D. (Group Leader, Neurotoxicology).

Key personnel participating in the conduct of the study were John F. Barnett, B.S. (Director of Laboratory Operations), Ronald J. McCarty, B.S. (Assistant Director of Laboratory Operations), Margaret M. Martin (Research Assistant/Assistant Supervisor), Susan L. DeHaven, B.S., Janet Dell, B.A., Elizabeth A. Jenkins, B.S., George K. Bentzel, B.S. and Jacqueline Manigault, B.S. (Laboratory Associates), Christopher S. Rutt (Animal Care Technician), Margie McMoore, B.S. (Information Specialist), James W. Bray, B.A. (Senior Information Specialist), Cheryl L. Van, B.A. (Methods Writer) and Cindy J. White (Senior Administrative Assistant). Additional personnel participating in the conduct of the study are identified in the raw data.

Curricula vitae and training records of personnel involved in the study are on file at Argus Research Laboratories, Inc.

3. Test Substance and Vehicle Identifications

Acrylamide (Sigma Chemical Co., Lot 50H0130), a white crystalline solid, was received at the Test Facility on July 26, 1991 and stored at room temperature, protected from light.

Carbaryl (Chem Service, Lot 56-98A) and Triadimefon (Chem Service, Lot 46-114A), both white powders, were received at the Test Facility on May 29, 1991 and stored at room temperature.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 2): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY - PREDOSAGE EXAMINATION

GROUP		MALES	FEMALES
RATS TESTED	N	28	28
URINE POOLS	MEAN $\pm$ S.D.	2.1 $\pm$ 1.8	0.7 $\pm$ 1.0**
LEVEL OF AROUSAL			
(1) Very low (stuporous)	N	0	0
(2) Low (sluggish)	N	0	0
(3) Apparently normal	N	28	28
(4) High (sudden startle)	N	0	0
(5) Very high (sudden running, vocalization)	N	0	0
	MEAN	3.0	3.0
BEHAVIORAL OR POSTURAL ALTERATIONS a			
None	N	28	26
Stereotyped behavior	N	0	0
Bizarre behavior	N	0	0
Twitches or tremors in limbs	N	0	0
Whole body tremors or spasms	N	0	2
Unusual posture	N	0	0
Tonic-clonic seizure	N	0	0
GAIT PATTERN			
Apparently normal	N	28	28
Ataxic	N	0	0
Exaggerated movements (limbs splayed)	N	0	0
Spastic, tip-toe	N	0	0
Duck-walk	N	0	0
Scissors gait	N	0	0
SEVERITY OF GAIT ABNORMALITY			
(1) Normal	N	28	28
(2) Slight	N	0	0
(3) Moderate	N	0	0
(4) Severe	N	0	0
	MEAN	1.0	1.0

(n) = Score assigned to graded test items.

a. Observation made in the open field.

\*\* Significantly different from the male group value ( $P \leq 0.01$ ).

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 3): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY - PREDOSAGE EXAMINATION

GROUP		MALES	FEMALES
RATS TESTED	N	28	28
PALPEBRAL CLOSURE			
(1) Wide open	N	28	28
(2) Slightly drooping	N	0	0
(3) Half closed	N	0	0
(4) Completely shut	N	0	0
	MEAN	1.0	1.0
PROMINENCE OF THE EYE			
Normal	N	28	28
Exophthalmos	N	0	0
Enophthalmos	N	0	0
LACRIMATION			
(1) No excess	N	28	28
(2) Excess at margin	N	0	0
(3) Persistent dampness	N	0	0
(4) Extends beyond margin	N	0	0
	MEAN	1.0	1.0
SALIVATION			
(1) No excess	N	28	28
(2) Slight wetness at margin	N	0	0
(3) Wetness 1/4 to 1/2 submaxillary	N	0	0
(4) Wetness over entire submaxillary	N	0	0
	MEAN	1.0	1.0
PILORECTION PRESENT	N	0	0
RESPIRATION ABNORMAL	N	0	0

(n) = Score assigned to graded test item.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 4): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY - PREDOSAGE EXAMINATION

GROUP		MALES	FEMALES
RATS TESTED	N	28	28
APPEARANCE			
(1) Clean and groomed	N	28	28
(2) Unkempt	N	0	0
(3) Stained by urine or feces	N	0	0
	MEAN	1.0	1.0
REACTION TO VISUAL STIMULUS			
(1) None	N	10	8
(2) Orienting	N	18	20
(3) Startle	N	0	0
(4) More energetic reaction	N	0	0
(5) Attacks	N	0	0
	MEAN	1.6	1.7
REACTION TO TACTILE STIMULUS			
(1) None	N	7	4
(2) Orienting	N	20	20
(3) Startle	N	1	4
(4) More energetic reaction	N	0	0
(5) Attacks	N	0	0
	MEAN	1.8	2.0
REACTION TO AUDITORY STIMULUS			
(1) None	N	0	0
(2) Orienting	N	6	4
(3) Startle	N	22	24
(4) More energetic reaction	N	0	0
(5) Attacks	N	0	0
	MEAN	2.8	2.8

(n) = Score assigned to graded test items.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 5): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY - PREDOSAGE EXAMINATION

GROUP		MALES	FEMALES
RATS TESTED	N	28	28
REACTION TO TAIL PINCH			
(1) None	N	2	2
(2) Orienting	N	11	18
(3) Startle	N	10	5
(4) More energetic reaction	N	5	3
(5) Attacks	N	0	0
	MEAN	2.6	2.3
VISUAL PLACING RESPONSE			
(1) Early extension	N	27	27
(2) Extension after contact	N	1	1
(3) No extension	N	0	0
	MEAN	1.0	1.0
AIR RIGHTING RESPONSE			
(1) Lands with all feet on the ground	N	24	27
(2) Uncoordinated landing	N	4	1
(3) Lands on back	N	0	0
	MEAN	1.1	1.0
NO PUPILLARY RESPONSE	N	0	0
GRIP-STRENGTH FORELIMB (G)			
Maximum	MEAN ± S.D.	854 ± 214	712 ± 204*
Average	MEAN ± S.D.	772 ± 198	634 ± 184**
GRIP-STRENGTH HINDLIMB (G)			
Maximum	MEAN ± S.D.	711 ± 193	586 ± 110**
Average	MEAN ± S.D.	635 ± 157	528 ± 101**
LANDING FOOT SPYAL AVERAGE (CM)	MEAN ± S.D.	7.6 ± 2.2	7.2 ± 1.7
BODY WEIGHT	MEAN ± S.D.	456 ± 46	293 ± 35**

(n) = Score assigned to graded test items.

\* Significantly different from the male group value (P<0.05).

\*\* Significantly different from the male group value (P<0.01).

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 1): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		I	III
DOSAGE (MG/KG/DAY) <sup>a</sup>		0 (VEHICLE, SALINE)	40 (ACRYLAMIDE)
<u>TEST SESSION 2b</u>			
RATS TESTED	N	8	8
HOME CAGE BEHAVIOR			
Sleeping	N	2	7*
Awake, Immobile	N	4	1
Normal movement	N	2	0
Unusual posture, tonic convulsion	N	0	0
Unusual behavior	N	0	0
BEHAVIORAL OR POSTURAL ALTERATIONS <sup>c</sup>			
None	N	8	8
Stereotyped behavior	N	0	0
Bizarre behavior	N	0	0
Twitches or tremors in limbs	N	0	0
Whole body tremors or spasms	N	0	0
Unusual posture	N	0	0
Tonic-clonic seizure	N	0	0
REACTION TO REMOVAL			
(1) Easily removed	N	8	6
(2) Vocalization, no resistance	N	0	0
(3) Runs or freezes (follows hand)	N	0	2
(4) Tail or throat rattles	N	0	0
	MEAN	1.0	1.5
REACTION TO HANDLING			
(1) Quiet, no resistance	N	8	5
(2) Vocalization, no resistance	N	0	0
(3) Tense	N	0	0
(4) Squirming	N	0	3
	MEAN	1.0	2.1
REARS (MALE)	MEAN ± S.D.	2.5 ± 1.7	1.2 ± 2.5
REARS (FEMALE)	MEAN ± S.D.	8.0 ± 6.3	0.0 ± 0.0*
FECAL BOLUSES (MALE)	MEAN ± S.D.	1.0 ± 1.4	0.2 ± 0.5
FECAL BOLUSES (FEMALE)	MEAN ± S.D.	0.5 ± 1.0	0.0 ± 0.0

(n) = Score assigned to graded test items.

a. Dosage occurred once daily for nine days.

b. Examination was conducted on day 7 of dosage.

c. Observation made at the home cage.

\* Significantly different from the vehicle control group value ( $P \leq 0.05$ ).

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 2): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		I	III
DOSAGE (MG/KG/DAY) <sup>a</sup>		0 (VEHICLE, SALINE)	40 (ACRYLAMIDE)
<u>TEST SESSION 2b</u>			
RATS TESTED	N	8	8
URINE POOLS (MALE)	MEAN $\pm$ S.D.	2.5 $\pm$ 2.1	1.0 $\pm$ 0.8
URINE POOLS (FEMALE)	MEAN $\pm$ S.D.	1.2 $\pm$ 1.0	0.8 $\pm$ 1.0
LEVEL OF AROUSAL			
(1) Very low (stuporous)	N	0	0
(2) Low (sluggish)	N	1	3
(3) Apparently normal	N	7	5
(4) High (sudden startle)	N	0	0
(5) Very high (sudden running, vocalization)	N	0	0
	MEAN	2.9	2.6
BEHAVIORAL OR POSTURAL ALTERATIONS <sup>c</sup>			
None	N	7	4
Stereotyped behavior	N	0	0
Bizarre behavior	N	0	0
Twitches or tremors in limbs	N	0	0
Whole body tremors or spasms	N	1	4
Unusual posture	N	0	0
Tonic-clonic seizure	N	0	0
GAIT PATTERN			
Apparently normal	N	8	4*
Ataxic	N	0	0
Exaggerated movements (limbs splayed)	N	0	4*
Spastic, tip-toe	N	0	0
Duck-walk	N	0	0
Scissors gait	N	0	0
SEVERITY OF GAIT ABNORMALITY			
(1) Normal	N	8	4
(2) Slight	N	0	3
(3) Moderate	N	0	1
(4) Severe	N	0	0
	MEAN	1.0	1.6*

(n) = Score assigned to graded test items.

a. Dosage occurred once daily for nine days.

b. Examination was conducted on day 7 of dosage.

c. Observation made at the home cage.

\* Significantly different from the vehicle control group value ( $P \leq 0.05$ ).

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 3): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		I	III
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, SALINE)	40(ACRYLAMIDE)
<u>TEST SESSION 2b</u>			
RATS TESTED	N	8	8
PALPEBRAL CLOSURE			
(1) Wide open	N	8	5
(2) Slightly drooping	N	0	3
(3) Half closed	N	0	0
(4) Completely shut	N	0	0
	MEAN	1.0	1.4
PROMINENCE OF THE EYE			
Normal	N	8	8
Exophthalmos	N	0	0
Enophthalmos	N	0	0
LACRIMATION			
(1) No excess	N	8	8
(2) Excess at margin	N	0	0
(3) Persistent dampness	N	0	0
(4) Extends beyond margin	N	0	0
	MEAN	1.0	1.0
SALIVATION			
(1) No excess	N	8	8
(2) Slight wetness at margin	N	0	0
(3) Wetness 1/4 to 1/2 submaxillary	N	0	0
(4) Wetness over entire submaxillary	N	0	0
	MEAN	1.0	1.0
PILOERECTOR PRESENT	N	0	0
RESPIRATION ABNORMAL	N	0	1

(n) = Score assigned to graded test item.

a. Dosage occurred once daily for nine days.

b. Examination was conducted on day 7 of dosage.



PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 4): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		I	III
DOSAGE (MG/KG/DAY)a		0(VEHICLE, SALINE)	40(ACRYLAMIDE)
<u>TEST SESSION 2b</u>			
RATS TESTED	N	8	8
APPEARANCE			
(1) Clean and groomed	N	8	8
(2) Unkempt	N	0	0
(3) Stained by urine or feces	N	0	0
	MEAN	1.0	1.0
REACTION TO VISUAL STIMULUS			
(1) None	N	2	4
(2) Orienting	N	6	4
(3) Startle	N	0	0
(4) More energetic reaction	N	0	0
(5) Attacks	N	0	0
	MEAN	1.8	1.5
REACTION TO TACTILE STIMULUS			
(1) None	N	2	1
(2) Orienting	N	6	7
(3) Startle	N	0	0
(4) More energetic reaction	N	0	0
(5) Attacks	N	0	0
	MEAN	1.8	1.9
REACTION TO AUDITORY STIMULUS			
(1) None	N	0	1
(2) Orienting	N	1	1
(3) Startle	N	7	6
(4) More energetic reaction	N	0	0
(5) Attacks	N	0	0
	MEAN	2.9	2.6

(n) = Score assigned to graded test items.

a. Dosage occurred once daily for nine days.

b. Examination was conducted on day 7 of dosage.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 5): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		I	III
DOSAGE (MG/KG/DAY)a		0(VEHICLE, SALINE)	40(ACRYLAMIDE)
<u>TEST SESSION 2b</u>			
RATS TESTED	N	8	8
REACTION TO TAIL PINCH			
(1) None	N	1	0
(2) Orienting	N	6	6
(3) Startle	N	0	1
(4) More energetic reaction	N	1	1
(5) Attacks	N	0	0
	MEAN	2.1	2.4
VISUAL PLACING RESPONSE			
(1) Early extension	N	8	8
(2) Extension after contact	N	0	0
(3) No extension	N	0	0
	MEAN	1.0	1.0
AIR RIGHTING RESPONSE			
(1) Lands with all feet on the ground	N	7	8
(2) Uncoordinated landing	N	1	0
(3) Lands on back	N	0	0
	MEAN	1.1	1.0
NO PUPILLARY RESPONSE			
	N	0	1
GRIP-STRENGTH FORELIMB (G)			
Maximum (MALE)	MEAN $\pm$ S.D.	1062 $\pm$ 196	788 $\pm$ 252
Maximum (FEMALE)	MEAN $\pm$ S.D.	750 $\pm$ 320	662 $\pm$ 165
Average (MALE)	MEAN $\pm$ S.D.	934 $\pm$ 148	741 $\pm$ 237
Average (FEMALE)	MEAN $\pm$ S.D.	597 $\pm$ 212	566 $\pm$ 137
GRIP-STRENGTH HINDLIMB (G)			
Maximum (MALE)	MEAN $\pm$ S.D.	756 $\pm$ 195	694 $\pm$ 149
Maximum (FEMALE)	MEAN $\pm$ S.D.	569 $\pm$ 97	462 $\pm$ 83
Average (MALE)	MEAN $\pm$ S.D.	703 $\pm$ 214	628 $\pm$ 136
Average (FEMALE)	MEAN $\pm$ S.D.	541 $\pm$ 103	431 $\pm$ 83
LANDING FOOT SPREAD AVERAGE (CM)	MEAN $\pm$ S.D.	7.3 $\pm$ 1.3	9.1 $\pm$ 2.8
BODY WEIGHT (MALE)	MEAN $\pm$ S.D.	449 $\pm$ 60	360 $\pm$ 33*
BODY WEIGHT (FEMALE)		289 $\pm$ 48	233 $\pm$ 30

(n) = Score assigned to graded test items.

a. Dosage occurred once daily for nine days.

b. Examination was conducted on day 7 of dosage.

\* Significantly different from the vehicle control group value ( $P \leq 0.05$ ).

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 6): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		I	III
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, SALINE)	40(ACRYLAMIDE)
<u>TEST SESSION 3b</u>			
RATS TESTED	N	8	8
HOME CAGE BEHAVIOR			
Sleeping	N	3	4
Awake, Immobile	N	3	3
Normal movement	N	2	0
Unusual posture, tonic convulsion	N	0	0
Unusual behavior	N	0	0
BEHAVIORAL OR POSTURAL ALTERATIONS <sup>c</sup>			
None	N	8	7
Stereotyped behavior	N	0	0
Bizarre behavior	N	0	0
Twitches or tremors in limbs	N	0	0
Whole body tremors or spasms	N	0	0
Unusual posture	N	0	0
Tonic-clonic seizure	N	0	0
REACTION TO REMOVAL			
(1) Easily removed	N	6	7
(2) Vocalization, no resistance	N	1	0
(3) Runs or freezes (follows hand)	N	1	0
(4) Tail or throat rattles	N	0	0
	MEAN	1.4	1.0
REACTION TO HANDLING			
(1) Quiet, no resistance	N	8	4
(2) Vocalization, no resistance	N	0	1
(3) Tense	N	0	2
(4) Squirming	N	0	0
	MEAN	1.0	1.5*
REARS (MALE)	MEAN ± S.D.	3.5 ± 1.9	0.2 ± 0.5*
REARS (FEMALE)	MEAN ± S.D.	7.2 ± 5.8	0.0 ± 0.0*
FECAL BOLUSES (MALE)	MEAN ± S.D.	0.0 ± 0.0	1.2 ± 1.9
FECAL BOLUSES (FEMALE)	MEAN ± S.D.	0.0 ± 0.0	1.5 ± 1.9

(n) = Score assigned to graded test items.

a. Dosage occurred once daily for nine days.

b. Examination was conducted on day 4 postdosage.

c. Observation made at the home cage.

\* Significantly different from the vehicle control group value ( $P \leq 0.05$ ).

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 7): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		I	III
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, SALINE)	40(ACRYLAMIDE)
<u>TEST SESSION 3b</u>			
RATS TESTED	N	8	8
URINE POOLS (MALE)	MEAN $\pm$ S.D.	2.2 $\pm$ 2.1	1.0 $\pm$ 1.7
URINE POOLS (FEMALE)	MEAN $\pm$ S.D.	0.2 $\pm$ 0.5	0.5 $\pm$ 0.6
LEVEL OF AROUSAL			
(1) Very low (stuporous)	N	0	0
(2) Low (sluggish)	N	1	5
(3) Apparently normal	N	7	3
(4) High (sudden startle)	N	0	0
(5) Very high (sudden running, vocalization)	N	0	0
	MEAN	2.9	2.4*
BEHAVIORAL OR POSTURAL ALTERATIONS <sup>c</sup>			
None	N	8	7
Stereotyped behavior	N	0	0
Bizarre behavior	N	0	0
Twitches or tremors in limbs	N	0	0
Whole body tremors or spasms	N	0	1
Unusual posture	N	0	0
Tonic-clonic seizure	N	0	0
GAIT PATTERN			
Apparently normal	N	8	0**
Ataxic	N	0	2
Exaggerated movements (limbs splayed)	N	0	6**
Spastic, tip-toe	N	0	0
Duck-walk	N	0	0
Scissors gait	N	0	0
SEVERITY OF GAIT ABNORMALITY			
(1) Normal	N	8	0
(2) Slight	N	0	0
(3) Moderate	N	0	5
(4) Severe	N	0	3
	MEAN	1.0	3.4**

(n) = Score assigned to graded test items.

a. Dosage occurred once daily for nine days.

b. Examination was conducted on day 4 postdosage.

c. Observation made in the open field.

\* Significantly different from the vehicle control group value ( $P < 0.05$ ).

\*\* Significantly different from the vehicle control group value ( $P \leq 0.01$ ).

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 8): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		I	III
DOSAGE (MG/KG/DAY)a		0(VEHICLE, SALINE)	40(ACRYLAMIDE)
<u>TEST SESSION 3b</u>			
RATS TESTED	N	8	8
PALPEBRAL CLOSURE			
(1) Wide open	N	8	4
(2) Slightly drooping	N	0	3
(3) Half closed	N	0	1
(4) Completely shut	N	0	0
	MEAN	1.0	1.6*
PROMINENCE OF THE EYE			
Normal	N	8	8
Exophthalmos	N	0	0
Enophthalmos	N	0	0
LACRIMATION			
(1) No excess	N	8	8
(2) Excess at margin	N	0	0
(3) Persistent dampness	N	0	0
(4) Extends beyond margin	N	0	0
	MEAN	1.0	1.0
SALIVATION			
(1) No excess	N	8	8
(2) Slight wetness at margin	N	0	0
(3) Wetness 1/4 to 1/2 submaxillary	N	0	0
(4) Wetness over entire submaxillary	N	0	0
	MEAN	1.0	1.0
PILOERECTION PRESENT	N	0	0
RESPIRATION ABNORMAL	N	1	5*

(n) = Score assigned to graded test item.

a. Dosage occurred once daily for nine days.

b. Examination was conducted on day 4 postdosage.

\* Significantly different from the vehicle control group value ( $P \leq 0.05$ ).

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 9): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		I	III
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, SALINE)	40(ACRYLAMIDE)
<u>TEST SESSION 3b</u>			
RATS TESTED	N	8	8
APPEARANCE			
(1) Clean and groomed	N	8	8
(2) Unkempt	N	0	0
(3) Stained by urine or feces	N	0	0
	MEAN	1.0	1.0
REACTION TO VISUAL STIMULUS			
(1) None	N	1	7
(2) Orienting	N	7	1
(3) Startle	N	0	0
(4) More energetic reaction	N	0	0
(5) Attacks	N	0	0
	MEAN	1.9	1.1**
REACTION TO TACTILE STIMULUS			
(1) None	N	2	4
(2) Orienting	N	6	4
(3) Startle	N	0	0
(4) More energetic reaction	N	0	0
(5) Attacks	N	0	0
	MEAN	1.8	1.5
REACTION TO AUDITORY STIMULUS			
(1) None	N	1	0
(2) Orienting	N	3	2
(3) Startle	N	4	6
(4) More energetic reaction	N	0	0
(5) Attacks	N	0	0
	MEAN	2.4	2.8

(n) = Score assigned to graded test items.

a. Dosage occurred once daily for nine days.

b. Examination was conducted on day 4 postdosage.

\*\* Significantly different from the vehicle control group value ( $P \leq 0.01$ ).

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 10): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		I	III
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, SALINE)	40(ACRYLAMIDE)
<u>TEST SESSION 3b</u>			
RATS TESTED	N	8	8
REACTION TO TAIL PINCH			
(1) None	N	2	1
(2) Orienting	N	3	5
(3) Startle	N	1	0
(4) More energetic reaction	N	2	2
(5) Attacks	N	0	0
	MEAN	2.4	2.4
VISUAL PLACING RESPONSE			
(1) Early extension	N	8	6
(2) Extension after contact	N	0	2
(3) No extension	N	0	0
	MEAN	1.0	1.2
AIR RIGHTING RESPONSE			
(1) Lands with all feet on the ground	N	7	4
(2) Uncoordinated landing	N	1	4
(3) Lands on back	N	0	0
	MEAN	1.1	1.5
NO PUPILLARY RESPONSE <sup>c</sup>	N	0	2
GRIP-STRENGTH FORELIMB (G)			
Maximum (MALE)	MEAN ± S.D.	919 ± 114	638 ± 246
Maximum (FEMALE)	MEAN ± S.D.	662 ± 192	469 ± 139
Average (MALE)	MEAN ± S.D.	859 ± 166	556 ± 210
Average (FEMALE)	MEAN ± S.D.	606 ± 232	422 ± 138
GRIP-STRENGTH HINDLIMB (G)			
Maximum (MALE)	MEAN ± S.D.	825 ± 378	800 ± 422
Maximum (FEMALE)	MEAN ± S.D.	575 ± 102	388 ± 111*
Average (MALE)	MEAN ± S.D.	709 ± 248	750 ± 426
Average (FEMALE)	MEAN ± S.D.	519 ± 111	341 ± 123
LANDING FOOT SPREAD AVERAGE (CM)	MEAN ± S.D.	6.3 ± 1.1	11.7 ± 1.4**
BODY WEIGHT (MALE)	MEAN ± S.D.	462 ± 62	377 ± 29*
BODY WEIGHT (FEMALE)	MEAN ± S.D.	298 ± 49	248 ± 39

(n) = Score assigned to graded test items.

a. Dosage occurred once daily for nine days.

b. Examination was conducted on day 4 postdosage.

c. Miosis occurred in 0/8 rats in Group I and 2/8 rats in Group III.

\* Significantly different from the vehicle control group value (P<0.05).

\*\* Significantly different from the vehicle control group value (P≤0.01).

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 1): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		I	IV
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, SALINE)	200(IDPN)
<u>TEST SESSION 2b</u>			
RATS TESTED	N	8	8
HOME CAGE BEHAVIOR			
Sleeping	N	2	7*
Awake, Immobile	N	4	1
Normal movement	N	2	0
Unusual posture, tonic convulsion	N	0	0
Unusual behavior	N	0	0
BEHAVIORAL OR POSTURAL ALTERATIONS <sup>c</sup>			
None	N	8	8
Stereotyped behavior	N	0	0
Bizarre behavior	N	0	0
Twitches or tremors in limbs	N	0	0
Whole body tremors or spasms	N	0	0
Unusual posture	N	0	0
Tonic-clonic seizure	N	0	0
REACTION TO REMOVAL			
(1) Easily removed	N	8	8
(2) Vocalization, no resistance	N	0	0
(3) Runs or freezes (follows hand)	N	0	0
(4) Tail or throat rattles	N	0	0
	MEAN	1.0	1.0
REACTION TO HANDLING			
(1) Quiet, no resistance	N	8	7
(2) Vocalization, no resistance	N	0	1
(3) Tense	N	0	0
(4) Squirming	N	0	0
	MEAN	1.0	1.1
REARS (MALE)	MEAN ± S.D.	2.5 ± 1.7	3.5 ± 1.3
REARS (FEMALE)	MEAN ± S.D.	8.0 ± 6.3	3.0 ± 3.5
FECAL BOLUSES (MALE)	MEAN ± S.D.	1.0 ± 1.4	0.5 ± 1.0
FECAL BOLUSES (FEMALE)	MEAN ± S.D.	0.5 ± 1.0	0.0 ± 0.0

(n) = Score assigned to graded test items.

a. Dosage occurred once daily for nine days in Group I and once daily for three days in Group IV.

b. The examination was conducted before dosage on day 7 of dosage in Group I and day 4 postdosage in Group IV.

c. Observation made at the home cage.

\* Significantly different from the vehicle control group value (P<0.05).



PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 2): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		I	IV
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, SALINE)	200(IDPM)
<u>TEST SESSION 2b</u>			
RATS TESTED	N	8	8
URINE POOLS (MALE)	MEAN + S.D.	2.5 + 2.1	1.8 + 1.2
URINE POOLS (FEMALE)	MEAN + S.D.	1.2 + 1.0	0.8 + 1.0
LEVEL OF AROUSAL			
(1) Very low (stuporous)	N	0	0
(2) Low (sluggish)	N	1	1
(3) Apparently normal	N	7	7
(4) High (sudden startle)	N	0	0
(5) Very high (sudden running, vocalization)	N	0	0
	MEAN	2.9	2.9
BEHAVIORAL OR POSTURAL ALTERATIONS <sup>c</sup>			
None	N	7	4
Stereotyped behavior	N	0	4*
Bizarre behavior	N	0	0
Twitches or tremors in limbs	N	0	0
Whole body tremors or spasms	N	1	0
Unusual posture	N	0	0
Tonic-clonic seizure	N	0	0
GAIT PATTERN			
Apparently normal	N	8	6
Ataxic	N	0	2
Exaggerated movements (limbs splayed)	N	0	0
Spastic, tip-toe	N	0	0
Duck-walk	N	0	0
Scissors gait	N	0	0
SEVERITY OF GAIT ABNORMALITY			
(1) Normal	N	8	6
(2) Slight	N	0	2
(3) Moderate	N	0	0
(4) Severe	N	0	0
	MEAN	1.0	1.2

(n) - Score assigned to graded test items.

a. Dosage occurred once daily for nine days in Group I and once daily for three days in Group IV.

b. The examination was conducted before dosage on day 7 of dosage in Group I and day 4 postdosage in Group IV.

c. Observation made in the open field.

\* Significantly different from the vehicle control group value ( $P \leq 0.05$ ).

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 3): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		I	IV
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, SALINE)	200(IDPN)
<u>TEST SESSION 2b</u>			
RATS TESTED	N	8	8
PALPEBRAL CLOSURE			
(1) Wide open	N	8	8
(2) Slightly drooping	N	0	0
(3) Half closed	N	0	0
(4) Completely shut	N	0	0
	MEAN	1.0	1.0
PROMINENCE OF THE EYE			
Normal	N	8	8
Exophthalmos	N	0	0
Enophthalmos	N	0	0
LACRIMATION			
(1) No excess	N	8	8
(2) Excess at margin	N	0	0
(3) Persistent dampness	N	0	0
(4) Extends beyond margin	N	0	0
	MEAN	1.0	1.0
SALIVATION			
(1) No excess	N	8	8
(2) Slight wetness at margin	N	0	0
(3) Wetness 1/4 to 1/2 submaxillary	N	0	0
(4) Wetness over entire submaxillary	N	0	0
	MEAN	1.0	1.0
PILOERECTOR PRESENT	N	0	0
RESPIRATION ABNORMAL	N	0	0

(n) = Score assigned to graded test item.

a. Dosage occurred once daily for nine days in Group I and once daily for three days in Group IV.

b. The examination was conducted before dosage on day 7 of dosage in Group I and day 4 postdosage in Group IV.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 4): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		I	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE, SALINE)	200(IDPN)
<u>TEST SESSION 2b</u>			
RATS TESTED	N	8	8
APPEARANCE			
(1) Clean and groomed	N	8	8
(2) Unkempt	N	0	0
(3) Stained by urine or feces	N	0	0
	MEAN	1.0	1.0
REACTION TO VISUAL STIMULUS			
(1) None	N	2	2
(2) Orienting	N	6	6
(3) Startle	N	0	0
(4) More energetic reaction	N	0	0
(5) Attacks	N	0	0
	MEAN	1.8	1.8
REACTION TO TACTILE STIMULUS			
(1) None	N	2	4
(2) Orienting	N	6	4
(3) Startle	N	0	0
(4) More energetic reaction	N	0	0
(5) Attacks	N	0	0
	MEAN	1.8	1.5
REACTION TO AUDITORY STIMULUS			
(1) None	N	0	3
(2) Orienting	N	1	1
(3) Startle	N	7	4
(4) More energetic reaction	N	0	0
(5) Attacks	N	0	0
	MEAN	2.9	2.1

(n) = Score assigned to graded test items.

a. Dosage occurred once daily for nine days in Group I and once daily for three days in Group IV.

b. The examination was conducted before dosage on day 7 of dosage in Group I and day 4 postdosage in Group IV.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 5): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		I	IV
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, SALINE)	200(IDPN)
<u>TEST SESSION 2b</u>			
RATS TESTED	N	8	8
REACTION TO TAIL PINCH			
(1) None	N	1	1
(2) Orienting	N	6	5
(3) Startle	N	0	0
(4) More energetic reaction	N	1	2
(5) Attacks	N	0	0
	MEAN	2.1	2.4
VISUAL PLACING RESPONSE			
(1) Early extension	N	8	8
(2) Extension after contact	N	0	0
(3) No extension	N	0	0
	MEAN	1.0	1.0
AIR RIGHTING RESPONSE			
(1) Lands with all feet on the ground	N	7	5
(2) Uncoordinated landing	N	1	3
(3) Lands on back	N	0	0
	MEAN	1.1	1.4
NO PUPILLARY RESPONSE <sup>c</sup>	N	0	1
GRIP-STRENGTH FORELIMB (G)			
Maximum (MALE)	MEAN ± S.D.	1062 ± 196	856 ± 363
Maximum (FEMALE)	MEAN ± S.D.	750 ± 320	662 ± 302
Average (MALE)	MEAN ± S.D.	934 ± 148	778 ± 308
Average (FEMALE)	MEAN ± S.D.	597 ± 212	559 ± 247
GRIP-STRENGTH HINDLIMB (G)			
Maximum (MALE)	MEAN ± S.D.	756 ± 195	669 ± 199
Maximum (FEMALE)	MEAN ± S.D.	569 ± 96	706 ± 103
Average (MALE)	MEAN ± S.D.	703 ± 214	609 ± 199
Average (FEMALE)	MEAN ± S.D.	541 ± 103	609 ± 146
LANDING FOOT SPREAD AVERAGE (CM)	MEAN ± S.D.	7.3 ± 1.3	8.3 ± 2.6
BODY WEIGHT (MALE)	MEAN ± S.D.	449 ± 60	364 ± 59
BODY WEIGHT (FEMALE)	MEAN ± S.D.	289 ± 48	260 ± 26

(n) = Score assigned to graded test items.

a. Dosage occurred once daily for nine days in Group I and once daily for three days in Group IV.

b. The examination was conducted before dosage on day 7 of dosage in Group I and day 4 postdosage in Group IV.

c. Miosis occurred in 0/8 rats in Group I and 1/8 rats in Group IV.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 6): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		I	IV
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, SALINE)	200(IDPN)
<u>TEST SESSION 3b</u>			
RATS TESTED	N	8	8
HOME CAGE BEHAVIOR			
Sleeping	N	3	5
Awake, Immobile	N	3	2
Normal movement	N	2	1
Unusual posture, tonic convulsion	N	0	0
Unusual behavior	N	0	0
BEHAVIORAL OR POSTURAL ALTERATIONS <sup>c</sup>			
None	N	8	7
Stereotyped behavior	N	0	1
Bizarre behavior	N	0	0
Twitches or tremors in limbs	N	0	0
Whole body tremors or spasms	N	0	0
Unusual posture	N	0	0
Tonic-clonic seizure	N	0	0
REACTION TO REMOVAL			
(1) Easily removed	N	6	8
(2) Vocalization, no resistance	N	1	0
(3) Runs or freezes (follows hand)	N	1	0
(4) Tail or throat rattles	N	0	0
	MEAN	1.4	1.0
REACTION TO HANDLING			
(1) Quiet, no resistance	N	8	8
(2) Vocalization, no resistance	N	0	0
(3) Tense	N	0	0
(4) Squirming	N	0	0
	MEAN	1.0	1.0
REARS (MALE)	MEAN ± S.D.	3.5 ± 1.9	2.0 ± 1.8
REARS (FEMALE)	MEAN ± S.D.	7.2 ± 5.8	3.2 ± 3.3
FECAL BOLUSES (MALE)	MEAN ± S.D.	0.0 ± 0.0	2.2 ± 1.7*
FECAL BOLUSES (FEMALE)	MEAN ± S.D.	0.0 ± 0.0	1.2 ± 1.9

(n) = Score assigned to graded test items.

a. Dosage occurred once daily for nine days in Group I and once daily for three days in Group IV.

b. The examination was conducted before dosage on day 4 postdosage in Group I and day 10 postdosage in Group IV.

c. Observation made at the home cage.

\* Significantly different from the vehicle control group value (P<0.05).

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Crl:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 7): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		I	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE, SALINE)	200(IDPM)
<u>TEST SESSION 3b</u>			
RATS TESTED	N	8	8
URINE POOLS (MALE)	MEAN $\pm$ S.D.	2.2 $\pm$ 2.1	2.5 $\pm$ 1.3
URINE POOLS (FEMALE)	MEAN $\pm$ S.D.	0.2 $\pm$ 0.5	1.0 $\pm$ 0.8
LEVEL OF AROUSAL			
(1) Very low (stuporous)	N	0	0
(2) Low (sluggish)	N	1	1
(3) Apparently normal	N	7	7
(4) High (sudden startle)	N	0	0
(5) Very high (sudden running, vocalization)	N	0	0
	MEAN	2.9	2.9
BEHAVIORAL OR POSTURAL ALTERATIONS c			
None	N	8	5
Stereotyped behavior	N	0	3
Bizarre behavior	N	0	1
Twitches or tremors in limbs	N	0	0
Whole body tremors or spasms	N	0	0
Unusual posture	N	0	0
Tonic-clonic seizure	N	0	0
GAIT PATTERN			
Apparently normal	N	8	7
Ataxic	N	0	1
Exaggerated movements (limbs splayed)	N	0	0
Spastic, tip-toe	N	0	0
Duck-walk	N	0	0
Scissors gait	N	0	0
SEVERITY OF GAIT ABNORMALITY			
(1) Normal	N	8	7
(2) Slight	N	0	1
(3) Moderate	N	0	0
(4) Severe	N	0	0
	MEAN	1.0	1.1

(n) = Score assigned to graded test items.

a. Dosage occurred once daily for nine days in Group I and once daily for three days in Group IV.

b. The examination was conducted before dosage on day 4 postdosage in Group I and day 10 postdosage in Group IV.

c. Observation made in the open field.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 8): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		I	IV
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, SALINE)	200(IDPN)
<u>TEST SESSION 3b</u>			
RATS TESTED	N	8	8
PALPEBRAL CLOSURE			
(1) Wide open	N	8	7
(2) Slightly drooping	N	0	1
(3) Half closed	N	0	0
(4) Completely shut	N	0	0
	MEAN	1.0	1.1
PROMINENCE OF THE EYE			
Normal	N	8	8
Exophthalmos	N	0	0
Enophthalmos	N	0	0
LACRIMATION			
(1) No excess	N	8	8
(2) Excess at margin	N	0	0
(3) Persistent dampness	N	0	0
(4) Extends beyond margin	N	0	0
	MEAN	1.0	1.0
SALIVATION			
(1) No excess	N	8	8
(2) Slight wetness at margin	N	0	0
(3) Wetness 1/4 to 1/2 submaxillary	N	0	0
(4) Wetness over entire submaxillary	N	0	0
	MEAN	1.0	1.0
PILOERECTION PRESENT	N	0	0
RESPIRATION ABNORMAL	N	1	1

(n) = Score assigned to graded test item.

a. Dosage occurred once daily for nine days in Group I and once daily for three days in Group IV.

b. The examination was conducted before dosage on day 4 postdosage in Group I and day 10 postdosage in Group IV.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 9): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		I	IV
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, SALINE)	200(IDPN)
<u>TEST SESSION 3b</u>			
RATS TESTED	N	8	8
APPEARANCE			
(1) Clean and groomed	N	8	8
(2) Unkempt	N	0	0
(3) Stained by urine or feces	N	0	0
	MEAN	1.0	1.0
REACTION TO VISUAL STIMULUS			
(1) None	N	1	1
(2) Orienting	N	7	7
(3) Startle	N	0	0
(4) More energetic reaction	N	0	0
(5) Attacks	N	0	0
	MEAN	1.9	1.9
REACTION TO TACTILE STIMULUS			
(1) None	N	2	3
(2) Orienting	N	6	5
(3) Startle	N	0	0
(4) More energetic reaction	N	0	0
(5) Attacks	N	0	0
	MEAN	1.6	1.6
REACTION TO AUDITORY STIMULUS			
(1) None	N	1	2
(2) Orienting	N	3	2
(3) Startle	N	4	4
(4) More energetic reaction	N	0	0
(5) Attacks	N	0	0
	MEAN	2.4	2.2

(n) = Score assigned to graded test items.

a. Dosage occurred once daily for nine days in Group I and once daily for three days in Group IV.

b. The examination was conducted before dosage on day 4 postdosage in Group I and day 10 postdosage in Group IV.



PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Crl:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 10): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		I	IV
DOSAGE (MG/KG/DAY) <sup>a</sup>		0 (VEHICLE, SALINE)	200 (IDPN)
<u>TEST SESSION 3b</u>			
RATS TESTED	N	8	8
REACTION TO TAIL PINCH			
(1) None	N	2	1
(2) Orienting	N	3	5
(3) Startle	N	1	1
(4) More energetic reaction	N	2	1
(5) Attacks	N	0	0
	MEAN	2.4	2.2
VISUAL PLACING RESPONSE			
(1) Early extension	N	8	7
(2) Extension after contact	N	0	1
(3) No extension	N	0	0
	MEAN	1.0	1.1
AIR RIGHTING RESPONSE			
(1) Lands with all feet on the ground	N	7	5
(2) Uncoordinated landing	N	1	2
(3) Lands on back	N	0	1
	MEAN	1.1	1.5
NO PUPILLARY RESPONSE <sup>c</sup>	N	0	1
GRIP-STRENGTH FORELIMB (G)			
Maximum (MALE)	MEAN ± S.D.	918 ± 114	656 ± 210
Maximum (FEMALE)	MEAN ± S.D.	662 ± 192	519 ± 194
Average (MALE)	MEAN ± S.D.	859 ± 166	600 ± 170
Average (FEMALE)	MEAN ± S.D.	606 ± 232	488 ± 180
GRIP-STRENGTH HINDLIMB (G)			
Maximum (MALE)	MEAN ± S.D.	825 ± 378	656 ± 216
Maximum (FEMALE)	MEAN ± S.D.	575 ± 102	606 ± 109
Average (MALE)	MEAN ± S.D.	709 ± 248	606 ± 189
Average (FEMALE)	MEAN ± S.D.	519 ± 111	556 ± 95
LANDING FOOT SPREAD AVERAGE (CM)	MEAN ± S.D.	6.3 ± 1.1	8.1 ± 3.1
BODY WEIGHT (MALE)	MEAN ± S.D.	462 ± 62	388 ± 73
BODY WEIGHT (FEMALE)	MEAN ± S.D.	298 ± 49	268 ± 32

(n) = Score assigned to graded test items.

a. Dosage occurred once daily for nine days in Group I and once daily for three days in Group IV.

b. The examination was conducted before dosage on day 4 postdosage in Group I and day 10 postdosage in Group IV.

c. Miosis occurred in 0/8 rats in Group I and 1/8 rats in Group IV.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 1): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		II	V
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, CORN OIL)	75(CARBARYL)
<u>TEST SESSION 2b</u>			
RATS TESTED	N	8	8
HOME CAGE BEHAVIOR			
Sleeping	N	3	2
Awake, immobile	N	5	5
Normal movement	N	0	1
Unusual posture, tonic convulsion	N	0	0
Unusual behavior	N	0	0
BEHAVIORAL OR POSTURAL ALTERATIONS <sup>c</sup>			
None	N	8	5
Stereotyped behavior	N	0	0
Bizarre behavior	N	0	0
Twitches or tremors in limbs	N	0	2
Whole body tremors or spasms	N	0	1
Unusual posture	N	0	0
Tonic-clonic seizure	N	0	0
REACTION TO REMOVAL			
(1) Easily removed	N	7	8
(2) Vocalization, no resistance	N	0	0
(3) Runs or freezes (follows hand)	N	1	0
(4) Tail or throat rattles	N	0	0
	MEAN	1.2	1.0
REACTION TO HANDLING			
(1) Quiet, no resistance	N	8	8
(2) Vocalization, no resistance	N	0	0
(3) Tense	N	0	0
(4) Squirming	N	0	0
	MEAN	1.0	1.0
REARS (MALE)	MEAN ± S.D.	0.5 ± 0.6	0.2 ± 0.5
REARS (FEMALE)	MEAN ± S.D.	0.2 ± 0.5	0.5 ± 1.0
FECAL BOLUSES (MALE)	MEAN ± S.D.	1.0 ± 2.0	0.0 ± 0.0
FECAL BOLUSES (FEMALE)	MEAN ± S.D.	1.2 ± 2.5	0.0 ± 0.0

(n) = Score assigned to graded test items.

a. Dosage occurred once.

b. The examination was conducted approximately 5.5 hours postdosage in Group II and 1 hour postdosage in Group V.

c. Observation made at the home cage.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 1): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		II	V
DOSAGE (MG/KG/DAY) <sup>a</sup>		0 (VEHICLE, CORN OIL)	75 (CARBARYL)
<u>TEST SESSION 2b</u>			
RATS TESTED	N	8	8
HOME CAGE BEHAVIOR			
Sleeping	N	3	2
Awake, Immobile	N	5	5
Normal movement	N	0	1
Unusual posture, tonic convulsion	N	0	0
Unusual behavior	N	0	0
BEHAVIORAL OR POSTURAL ALTERATIONS <sup>c</sup>			
None	N	8	5
Stereotyped behavior	N	0	0
Bizarre behavior	N	0	0
Twitches or tremors in limbs	N	0	2
Whole body tremors or spasms	N	0	1
Unusual posture	N	0	0
Tonic-clonic seizure	N	0	0
REACTION TO REMOVAL			
(1) Easily removed	N	7	8
(2) Vocalization, no resistance	N	0	0
(3) Runs or freezes (follows hand)	N	1	0
(4) Tail or throat rattles	N	0	0
	MEAN	1.2	1.0
REACTION TO HANDLING			
(1) Quiet, no resistance	N	8	8
(2) Vocalization, no resistance	N	0	0
(3) Tense	N	0	0
(4) Squirming	N	0	0
	MEAN	1.0	1.0
REARS (MALE)	MEAN ± S.D.	0.5 ± 0.6	0.2 ± 0.5
REARS (FEMALE)	MEAN ± S.D.	0.2 ± 0.5	0.5 ± 1.0
FECAL BOLUSES (MALE)	MEAN ± S.D.	1.0 ± 2.0	0.0 ± 0.0
FECAL BOLUSES (FEMALE)	MEAN ± S.D.	1.2 ± 2.5	0.0 ± 0.0

(n) = Score assigned to graded test items.

a. Dosage occurred once.

b. The examination was conducted approximately 5.5 hours postdosage in Group II and 1 hour postdosage in Group V.

c. Observation made at the home cage.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 2): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		II	V
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, CORN OIL)	75(CARBARYL)
<u>TEST SESSION 2b</u>			
RATS TESTED	N	8	8
URINE POOLS (MALE)	MEAN ± S.D.	1.0 ± 0.8	2.5 ± 1.3
URINE POOLS (FEMALE)	MEAN ± S.D.	1.0 ± 0.8	3.2 ± 1.9*
LEVEL OF AROUSAL			
(1) Very low (stuporous)	N	0	0
(2) Low (sluggish)	N	3	6
(3) Apparently normal	N	5	2
(4) High (sudden startle)	N	0	0
(5) Very high (sudden running, vocalization)	N	0	0
	MEAN	2.6	2.2
BEHAVIORAL OR POSTURAL ALTERATIONS <sup>c</sup>			
None	N	7	1**
Stereotyped behavior	N	0	0
Bizarre behavior	N	0	0
Twitches or tremors in limbs	N	0	7**
Whole body tremors or spasms	N	1	0
Unusual posture	N	0	0
Tonic-clonic seizure	N	0	0
GAIT PATTERN			
Apparently normal	N	8	6
Ataxic	N	0	0
Exaggerated movements (limbs splayed)	N	0	1
Spastic, tip-toe	N	0	1
Duck-walk	N	0	0
Scissors gait	N	0	0
SEVERITY OF GAIT ABNORMALITY			
(1) Normal	N	8	6
(2) Slight	N	0	0
(3) Moderate	N	0	2
(4) Severe	N	0	0
	MEAN	1.0	1.5

(n) = Score assigned to graded test items.

a. Dosage occurred once.

b. The examination was conducted approximately 5.5 hours postdosage in Group II and 1 hour postdosage in Group V.

c. Observation made in the open field.

\* Significantly different from the vehicle control group value (P<0.05).

\*\* Significantly different from the vehicle control group value (P<0.01).

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 3): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		II	V
DOSAGE (MG/KG/DAY) <sup>a</sup>		0 (VEHICLE, CORN OIL)	75 (CARBARYL)
<u>TEST SESSION 2b</u>			
RATS TESTED	N	8	8
PALPEBRAL CLOSURE			
(1) Wide open	N	3	8
(2) Slightly drooping	N	4	0
(3) Half closed	N	1	0
(4) Completely shut	N	0	0
	MEAN	1.8	1.0**
PROMINENCE OF THE EYE			
Normal	N	8	8
Exophthalmos	N	0	0
Enophthalmos	N	0	0
LACRIMATION			
(1) No excess	N	8	3
(2) Excess at margin	N	0	3
(3) Persistent dampness	N	0	2
(4) Extends beyond margin	N	0	0
	MEAN	1.0	1.9*
SALIVATION			
(1) No excess	N	8	2
(2) Slight wetness at margin	N	0	1
(3) Wetness 1/4 to 1/2 submaxillary	N	0	2
(4) Wetness over entire submaxillary	N	0	3
	MEAN	1.0	2.8**
PILOERECTION PRESENT	N	0	0
RESPIRATION ABNORMAL	N	2	3

(n) = Score assigned to graded test item.

a. Dosage occurred once.

b. The examination was conducted approximately 5.5 hours postdosage in Group II and 1 hour postdosage in Group V.

\* Significantly different from the vehicle control group value ( $P \leq 0.05$ ).

\*\* Significantly different from the vehicle control group value ( $P \leq 0.01$ ).

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Crl:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 4): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		II	V
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, CORN OIL)	75(CARBARYL)
<u>TEST SESSION 2b</u>			
RATS TESTED	N	8	8
APPEARANCE			
(1) Clean and groomed	N	8	7
(2) Unkempt	N	0	0
(3) Stained by urine or feces	N	0	1
	MEAN	1.0	1.2
REACTION TO VISUAL STIMULUS			
(1) None	N	4	6
(2) Orienting	N	3	2
(3) Startle	N	1	0
(4) More energetic reaction	N	0	0
(5) Attacks	N	0	0
	MEAN	1.6	1.2
REACTION TO TACTILE STIMULUS			
(1) None	N	4	6
(2) Orienting	N	4	2
(3) Startle	N	0	0
(4) More energetic reaction	N	0	0
(5) Attacks	N	0	0
	MEAN	1.5	1.2
REACTION TO AUDITORY STIMULUS			
(1) None	N	0	3
(2) Orienting	N	0	1
(3) Startle	N	8	4
(4) More energetic reaction	N	0	0
(5) Attacks	N	0	0
	MEAN	3.0	2.1*

(n) = Score assigned to graded test items.

a. Dosage occurred once.

b. The examination was conducted approximately 5.5 hours postdosage in Group II and 1 hour postdosage in Group V.

\* Significantly different from the vehicle control group value ( $P \leq 0.05$ ).

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 5): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		II	V
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, CORN OIL)	75(CARBARYL)
<u>TEST SESSION 2b</u>			
RATS TESTED	N	8	8
REACTION TO TAIL PINCH			
(1) None	N	1	7
(2) Orienting	N	4	1
(3) Startle	N	1	0
(4) More energetic reaction	N	2	0
(5) Attacks	N	0	0
	MEAN	2.5	1.1**
VISUAL PLACING RESPONSE			
(1) Early extension	N	8	3
(2) Extension after contact	N	0	3
(3) No extension	N	0	2
	MEAN	1.0	1.9**
AIR RIGHTING RESPONSE			
(1) Lands with all feet on the ground	N	7	3
(2) Uncoordinated landing	N	1	3
(3) Lands on back	N	0	2
	MEAN	1.1	1.9*
NO PUPILLARY RESPONSE <sup>c</sup>			
	N	1	4
GRIP-STRENGTH FORELIMB (G)			
Maximum (MALE)	MEAN ± S.D.	719 ± 109	712 ± 282
Maximum (FEMALE)	MEAN ± S.D.	706 ± 398	562 ± 190
Average (MALE)	MEAN ± S.D.	659 ± 89	594 ± 208
Average (FEMALE)	MEAN ± S.D.	628 ± 332	466 ± 148
GRIP-STRENGTH HINDLIMB (G)			
Maximum (MALE)	MEAN ± S.D.	800 ± 140	781 ± 335
Maximum (FEMALE)	MEAN ± S.D.	606 ± 120	731 ± 166
Average (MALE)	MEAN ± S.D.	734 ± 168	712 ± 288
Average (FEMALE)	MEAN ± S.D.	553 ± 89	625 ± 123
LANDING FOOT SPREAD AVERAGE (CM)	MEAN ± S.D.	7.0 ± 0.8	8.1 ± 1.9
BODY WEIGHT (MALE)	MEAN ± S.D.	486 ± 36	495 ± 45
BODY WEIGHT (FEMALE)	MEAN ± S.D.	310 ± 49	291 ± 46

(n) = Score assigned to graded test items.

a. Dosage occurred once.

b. The examination was conducted approximately 5.5 hours postdosage in Group II and 1 hour postdosage in Group V.

c. Miosis occurred in 0/8 rats in Group II and 5/8 rats in Group V.

\* Significantly different from the vehicle control group value ( $P \leq 0.05$ ).

\*\* Significantly different from the vehicle control group value ( $P \leq 0.01$ ).

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 1): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		II	VI
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, CORN OIL)	75(DDT)
<u>TEST SESSION 2b</u>			
RATS TESTED	N	8	8
HOME CAGE BEHAVIOR			
Sleeping	N	3	3
Awake, Immobile	N	5	4
Normal movement	N	0	1
Unusual posture, tonic convulsion	N	0	0
Unusual behavior	N	0	0
BEHAVIORAL OR POSTURAL ALTERATIONS <sup>c</sup>			
None	N	8	6
Stereotyped behavior	N	0	0
Bizarre behavior	N	0	0
Twitches or tremors in limbs	N	0	0
Whole body tremors or spasms	N	0	2
Unusual posture	N	0	0
Tonic-clonic seizure	N	0	0
REACTION TO REMOVAL			
(1) Easily removed	N	7	7
(2) Vocalization, no resistance	N	0	0
(3) Runs or freezes (follows hand)	N	1	1
(4) Tail or throat rattles	N	0	0
	MEAN	1.2	1.2
REACTION TO HANDLING			
(1) Quiet, no resistance	N	8	8
(2) Vocalization, no resistance	N	0	0
(3) Tense	N	0	0
(4) Squirming	N	0	0
	MEAN	1.0	1.0
REARS (MALE)	MEAN ± S.D.	0.5 ± 0.6	2.5 ± 1.3*
REARS (FEMALE)	MEAN ± S.D.	0.2 ± 0.5	0.2 ± 0.5
FECAL BOLUSES (MALE)	MEAN ± S.D.	1.0 ± 2.0	1.5 ± 1.7
FECAL BOLUSES (FEMALE)	MEAN ± S.D.	1.2 ± 2.5	0.2 ± 0.5

(n) = Score assigned to graded test items.

a. Dosage occurred once.

b. The examination was conducted approximately 5.5 hours postdosage.

c. Observation made at the home cage.

\* Significantly different from the vehicle control group value ( $P \leq 0.05$ ).



PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 2): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		II	VI
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, CORN OIL)	75(DDT)
<u>TEST SESSION 2b</u>			
RATS TESTED	N	8	8
URINE POOLS (MALE)	MEAN ± S.D.	1.0 ± 0.8	2.5 ± 1.0
URINE POOLS (FEMALE)		1.0 ± 0.8	1.8 ± 1.2
LEVEL OF AROUSAL			
(1) Very low (stuporous)	N	0	0
(2) Low (sluggish)	N	3	0
(3) Apparently normal	N	5	8
(4) High (sudden startle)	N	0	0
(5) Very high (sudden running, vocalization)	N	0	0
	MEAN	2.6	3.0
BEHAVIORAL OR POSTURAL ALTERATIONS <sup>c</sup>			
None	N	7	4
Stereotyped behavior	N	0	0
Bizarre behavior	N	0	0
Twitches or tremors in limbs	N	0	1
Whole body tremors or spasms	N	1	3
Unusual posture	N	0	0
Tonic-clonic seizure	N	0	0
GAIT PATTERN			
Apparently normal	N	8	8
Ataxic	N	0	0
Exaggerated movements (limbs splayed)	N	0	0
Spastic, tip-toe	N	0	0
Duck-walk	N	0	0
Scissors gait	N	0	0
SEVERITY OF GAIT ABNORMALITY			
(1) Normal	N	8	8
(2) Slight	N	0	0
(3) Moderate	N	0	0
(4) Severe	N	0	0
	MEAN	1.0	1.0

(n) = Score assigned to graded test items.

a. Dosage occurred once.

b. The examination was conducted approximately 5.5 hours postdosage.

c. Observation made in the open field.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 3): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		II	VI
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, CORN OIL)	75(DDT)
<u>TEST SESSION 2b</u>			
RATS TESTED	N	8	8
PALPEBRAL CLOSURE			
(1) Wide open	N	3	7
(2) Slightly drooping	N	4	1
(3) Half closed	N	1	0
(4) Completely shut	N	0	0
	MEAN	1.8	1.1*
PROMINENCE OF THE EYE			
Normal	N	8	8
Exophthalmos	N	0	0
Enophthalmos	N	0	0
LACRIMATION			
(1) No excess	N	8	8
(2) Excess at margin	N	0	0
(3) Persistent dampness	N	0	0
(4) Extends beyond margin	N	0	0
	MEAN	1.0	1.0
SALIVATION			
(1) No excess	N	8	8
(2) Slight wetness at margin	N	0	0
(3) Wetness 1/4 to 1/2 submaxillary	N	0	0
(4) Wetness over entire submaxillary	N	0	0
	MEAN	1.0	1.0
PILOERECTION PRESENT	N	0	0
RESPIRATION ABNORMAL	N	2	0

(n) = Score assigned to graded test item.

a. Dosage occurred once.

b. The examination was conducted approximately 5.5 hours postdosage.

\* Significantly different from the vehicle control group value ( $P \leq 0.05$ ).

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 4): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		II	VI
DOSAGE (MG/KG/DAY)a		0(VEHICLE, CORN OIL)	75(DDT)
<u>TEST SESSION 2b</u>			
RATS TESTED	N	8	8
APPEARANCE			
(1) Clean and groomed	N	8	8
(2) Unkempt	N	0	0
(3) Stained by urine or feces	N	0	0
	MEAN	1.0	1.0
REACTION TO VISUAL STIMULUS			
(1) None	N	4	4
(2) Orienting	N	3	4
(3) Startle	N	1	0
(4) More energetic reaction	N	0	0
(5) Attacks	N	0	0
	MEAN	1.6	1.5
REACTION TO TACTILE STIMULUS			
(1) None	N	4	4
(2) Orienting	N	4	4
(3) Startle	N	0	0
(4) More energetic reaction	N	0	0
(5) Attacks	N	0	0
	MEAN	1.5	1.5
REACTION TO AUDITORY STIMULUS			
(1) None	N	0	0
(2) Orienting	N	0	1
(3) Startle	N	8	7
(4) More energetic reaction	N	0	0
(5) Attacks	N	0	0
	MEAN	3.0	2.9

(n) = Score assigned to graded test items.

a. Dosage occurred once.

b. The examination was conducted approximately 5.5 hours postdosage.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 5): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		II	VI
DOSAGE (MG/KG/DAY) <sup>a</sup>		0 (VEHICLE, CORN OIL)	75 (DDT)
<u>TEST SESSION 2b</u>			
RATS TESTED	N	8	8
REACTION TO TAIL PINCH			
(1) None	N	1	0
(2) Orienting	N	4	5
(3) Startle	N	1	1
(4) More energetic reaction	N	2	2
(5) Attacks	N	0	0
	MEAN	2.5	2.6
VISUAL PLACING RESPONSE			
(1) Early extension	N	8	8
(2) Extension after contact	N	0	0
(3) No extension	N	0	0
	MEAN	1.0	1.0
AIR RIGHTING RESPONSE			
(1) Lands with all feet on the ground	N	7	7
(2) Uncoordinated landing	N	1	1
(3) Lands on back	N	0	0
	MEAN	1.1	1.1
NO PUPILLARY RESPONSE <sup>c</sup>	N	1	0
GRIP-STRENGTH FORELIMB (G)			
Maximum (MALE)	MEAN ± S.D.	719 ± 109	719 ± 247
Maximum (FEMALE)	MEAN ± S.D.	706 ± 398	631 ± 255
Average (MALE)	MEAN ± S.D.	659 ± 89	644 ± 215
Average (FEMALE)	MEAN ± S.D.	628 ± 332	531 ± 186
GRIP-STRENGTH HINDLIMB (G)			
Maximum (MALE)	MEAN ± S.D.	800 ± 140	656 ± 142
Maximum (FEMALE)	MEAN ± S.D.	606 ± 120	594 ± 180
Average (MALE)	MEAN ± S.D.	734 ± 168	591 ± 157
Average (FEMALE)	MEAN ± S.D.	553 ± 89	522 ± 124
LANDING FOOT SPREAD AVERAGE (CM)	MEAN ± S.D.	7.0 ± 0.8	7.5 ± 1.4
BODY WEIGHT (MALE)	MEAN ± S.D.	486 ± 36	452 ± 68
BODY WEIGHT (FEMALE)	MEAN ± S.D.	310 ± 49	295 ± 29

(n) = Score assigned to graded test items.

a. Dosage occurred once.

b. The examination was conducted approximately 5.5 hours postdosage.

c. Miosis occurred in 0/8 rats in Group II and 1/8 rats in Group VI.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 1): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		II	VII
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, CORN OIL)	TRIADIMEFON
<u>TEST SESSION 2b</u>			
RATS TESTED	N	8	8
HOME CAGE BEHAVIOR			
Sleeping	N	3	1
Awake, Immobile	N	5	2
Normal movement	N	0	5**
Unusual posture, tonic convulsion	N	0	0
Unusual behavior	N	0	0
BEHAVIORAL OR POSTURAL ALTERATIONS <sup>c</sup>			
None	N	8	8
Stereotyped behavior	N	0	0
Bizarre behavior	N	0	0
Twitches or tremors in limbs	N	0	0
Whole body tremors or spasms	N	0	0
Unusual posture	N	0	0
Tonic-clonic seizure	N	0	0
REACTION TO REMOVAL			
(1) Easily removed	N	7	6
(2) Vocalization, no resistance	N	0	0
(3) Runs or freezes (follows hand)	N	1	2
(4) Tail or throat rattles	N	0	0
	MEAN	1.2	1.5
REACTION TO HANDLING			
(1) Quiet, no resistance	N	8	6
(2) Vocalization, no resistance	N	0	0
(3) Tense	N	0	2
(4) Squirming	N	0	0
	MEAN	1.0	1.5
REARS (MALE)	MEAN ± S.D.	0.5 ± 0.6	0.5 ± 1.0
REARS (FEMALE)	MEAN ± S.D.	0.2 ± 0.5	4.5 ± 3.0*
FECAL BOLUSES (MALE)	MEAN ± S.D.	1.0 ± 2.0	2.5 ± 2.6
FECAL BOLUSES (FEMALE)	MEAN ± S.D.	1.2 ± 2.5	0.5 ± 1.0

(n) = Score assigned to graded test items.

a. Dosage occurred once.

b. The examination was conducted approximately 5.5 hours postdosage in Group II and 2 hours postdosage in Group VII.

c. Observation made at the home cage.

\* Significantly different from the vehicle control group value (P<0.05).

\*\* Significantly different from the vehicle control group value (p<0.01).

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 2): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		II	VII
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, CORN OIL)	TRIADIMEFON
<u>TEST SESSION 2b</u>			
RATS TESTED	N	8	8
URINE POOLS (MALE)	MEAN $\pm$ S.D.	1.0 $\pm$ 0.8	3.2 $\pm$ 2.6
URINE POOLS (FEMALE)	MEAN $\pm$ S.D.	1.0 $\pm$ 0.8	0.8 $\pm$ 1.0
LEVEL OF AROUSAL			
(1) Very low (stuporous)	N	0	0
(2) Low (sluggish)	N	3	0
(3) Apparently normal	N	5	8
(4) High (sudden startle)	N	0	0
(5) Very high (sudden running, vocalization)	N	0	0
	MEAN	2.6	3.0
BEHAVIORAL OR POSTURAL ALTERATIONS c			
None	N	7	8
Stereotyped behavior	N	0	0
Bizarre behavior	N	0	0
Twitches or tremors in limbs	N	0	0
Whole body tremors or spasms	N	1	0
Unusual posture	N	0	0
Tonic-clonic seizure	N	0	0
GAIT PATTERN			
Apparently normal	N	8	8
Ataxic	N	0	0
Exaggerated movements (limbs splayed)	N	0	0
Spastic, tip-toe	N	0	0
Duck-walk	N	0	0
Scissors gait	N	0	0
SEVERITY OF GAIT ABNORMALITY			
(1) Normal	N	8	8
(2) Slight	N	0	0
(3) Moderate	N	0	0
(4) Severe	N	0	0
	MEAN	1.0	1.0

(n) = Score assigned to graded test items.

a. Dosage occurred once.

b. The examination was conducted approximately 5.5 hours postdosage in Group II and 2 hours postdosage in Group VII.

c. Observation made in the open field.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 3): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		II	VII
DOSAGE (MG/KG/DAY) <sup>a</sup>		O(VEHICLE, CORN OIL)	TRIADIMEFON
<u>TEST SESSION 2b</u>			
RATS TESTED	N	8	8
PALPEBRAL CLOSURE			
(1) Wide open	N	3	8
(2) Slightly drooping	N	4	0
(3) Half closed	N	1	0
(4) Completely shut	N	0	0
	MEAN	1.8	1.0**
PROMINENCE OF THE EYE			
Normal	N	8	7
Exophthalmos	N	0	1
Enophthalmos	N	0	0
LACRIMATION			
(1) No excess	N	8	8
(2) Excess at margin	N	0	0
(3) Persistent dampness	N	0	0
(4) Extends beyond margin	N	0	0
	MEAN	1.0	1.0
SALIVATION			
(1) No excess	N	8	6
(2) Slight wetness at margin	N	0	1
(3) Wetness 1/4 to 1/2 submaxillary	N	0	0
(4) Wetness over entire submaxillary	N	0	1
	MEAN	1.0	1.5
PILOERECTION PRESENT	N	0	0
RESPIRATION ABNORMAL	N	2	0

(n) = Score assigned to graded test item.

a. Dosage occurred once.

b. The examination was conducted approximately 5.5 hours postdosage in Group II and 2 hours postdosage in Group VII.

\*\* Significantly different from the vehicle control group value ( $P \leq 0.01$ ).

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 4): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		II	VII
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, CORN OIL)	TRIADIMEFON
<u>TEST SESSION 2b</u>			
RATS TESTED	N	8	8
APPEARANCE			
(1) Clean and groomed	N	8	8
(2) Unkempt	N	0	0
(3) Stained by urine or feces	N	0	0
	MEAN	1.0	1.0
REACTION TO VISUAL STIMULUS			
(1) None	N	4	3
(2) Orienting	N	3	5
(3) Startle	N	1	0
(4) More energetic reaction	N	0	0
(5) Attacks	N	0	0
	MEAN	1.6	1.6
REACTION TO TACTILE STIMULUS			
(1) None	N	4	1
(2) Orienting	N	4	7
(3) Startle	N	0	0
(4) More energetic reaction	N	0	0
(5) Attacks	N	0	0
	MEAN	1.5	1.9
REACTION TO AUDITORY STIMULUS			
(1) None	N	0	0
(2) Orienting	N	0	0
(3) Startle	N	8	7
(4) More energetic reaction	N	0	1
(5) Attacks	N	0	0
	MEAN	3.0	3.1

(n) = Score assigned to graded test items.

a. Dosage occurred once.

b. The examination was conducted approximately 5.5 hours postdosage in Group II and 2 hours postdosage in Group VII.



PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 5): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY

DOSAGE GROUP		II	VII
DOSAGE (MG/KG/DAY)a		0(VEHICLE, CORN OIL)	TRIADIMEFON
<u>TEST SESSION 2b</u>			
RATS TESTED	N	8	8
REACTION TO TAIL PINCH			
(1) None	N	1	1
(2) Orienting	N	4	5
(3) Startle	N	1	0
(4) More energetic reaction	N	2	2
(5) Attacks	N	0	0
	MEAN	2.5	2.4
VISUAL PLACING RESPONSE			
(1) Early extension	N	8	8
(2) Extension after contact	N	0	0
(3) No extension	N	0	0
	MEAN	1.0	1.0
AIR RIGHTING RESPONSE			
(1) Lands with all feet on the ground	N	7	8
(2) Uncoordinated landing	N	1	0
(3) Lands on back	N	0	0
	MEAN	1.1	1.0
NO PUPILLARY RESPONSE c	N	1	0
GRIP-STRENGTH FORELIMB (G)			
Maximum (MALE)	MEAN ± S.D.	719 ± 109	800 ± 246
Maximum (FEMALE)	MEAN ± S.D.	706 ± 398	719 ± 213
Average (MALE)	MEAN ± S.D.	659 ± 89	753 ± 195
Average (FEMALE)	MEAN ± S.D.	628 ± 332	628 ± 221
GRIP-STRENGTH HINDLIMB (G)			
Maximum (MALE)	MEAN ± S.D.	800 ± 140	700 ± 172
Maximum (FEMALE)	MEAN ± S.D.	606 ± 120	538 ± 142
Average (MALE)	MEAN ± S.D.	734 ± 168	656 ± 198
Average (FEMALE)	MEAN ± S.D.	553 ± 89	509 ± 153
LANDING FOOT SPREAD AVERAGE (CM)	MEAN ± S.D.	7.0 ± 0.8	7.3 ± 1.4
BODY WEIGHT (MALE)	MEAN ± S.D.	486 ± 36	477 ± 35
BODY WEIGHT (FEMALE)	MEAN ± S.D.	310 ± 49	296 ± 43

(a) = Score assigned to graded test items.

a. Dosage occurred once.

b. The examination was conducted approximately 5.5 hours postdosage in Group II and 2 hours postdosage in Group VII.

c. Miosis occurred in 0/8 rats in Group II and 1/8 rats in Group VII.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 3 (PAGE 1): FUNCTIONAL OBSERVATIONAL BATTERY - INDIVIDUAL DATA - MALE RATS

PREDOSAGE EXAMINATION

RAT #	19761	19762	19782	19787	19765	19767	19777	19785	19764	19768	19770	19776	19775	19780	19784	19788
DOSAGE GROUP	I	I	I	I	II	II	II	II	III	III	III	III	IV	IV	IV	IV
HOME CAGE BEHAVIOR	1	1	3	2	1	2	3	2	1	1	1	2	2	2	1	1
BEHAVIORAL OR POSTURAL ALTERATIONS a	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
REACTION TO REMOVAL	1	1	3	2	2	2	1	1	1	1	1	2	2	2	1	2
REACTION TO HANDLING	1	1	3	1	1	3	1	1	1	1	1	2	1	1	1	4
REARS	1	1	7	0	2	4	1	0	1	2	1	0	0	3	0	2
FECAL BOLUSES	0	0	4	0	0	0	0	2	5	4	2	2	1	3	0	4
URINE POOLS	0	1	3	8	0	1	3	0	3	4	0	3	3	1	3	0
LEVEL OF AROUSAL	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
BEHAVIORAL OR POSTURAL ALTERATIONS b	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
GAIT PATTERN	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SEVERITY OF GAIT ABNORMALITY	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
PALPEBRAL CLOSURE	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
PROMINENCE OF THE EYE	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
LACRIMATION	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SALIVATION	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
PILOERECTION	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RESPIRATION	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
APPEARANCE	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
REACTION TO VISUAL STIMULUS	2	1	2	2	2	2	1	1	2	1	2	1	2	1	2	2
REACTION TO TACTILE STIMULUS	2	1	2	2	2	2	1	2	2	2	2	3	2	2	2	2
REACTION TO AUDITORY STIMULUS	3	3	3	3	3	3	3	3	2	3	2	3	3	3	3	2
REACTION TO TAIL PINCH	3	1	3	2	3	4	3	2	3	2	2	4	4	3	4	1
VISUAL PLACING RESPONSE	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
AIR RIGHTING RESPONSE	1	1	1	1	1	1	2	1	1	2	1	1	1	1	1	1
PUPILLARY RESPONSE	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
FORELIMB GRIP STRENGTH (G) #1	950	750	825	1050	575	925	1025	600	975	1225	775	1250	525	700	850	825
FORELIMB GRIP STRENGTH (G) #2	500	475	725	950	400	500	1200	625	900	525	775	1250	450	550	950	775
HINDLIMB GRIP STRENGTH (G) #1	475	650	425	350	775	500	500	750	450	875	775	725	425	725	775	800
HINDLIMB GRIP STRENGTH (G) #2	550	800	400	550	925	500	450	950	575	525	425	825	450	550	600	550
LANDING FOOT SPREAD (CM) #1	6.5	10.5	6.0	5.5	11.3	8.5	8.0	8.6	6.0	7.0	5.5	6.5	6.8	11.5	7.0	11.0
LANDING FOOT SPREAD (CM) #2	6.0	13.2	6.0	7.5	9.0	9.7	9.0	6.8	7.0	5.7	6.3	5.5	4.8	11.0	10.0	9.0
BODY WEIGHT	491	460	476	345	461	473	520	436	471	414	482	433	363	454	443	530

#1 = FIRST MEASUREMENT

#2 = SECOND MEASUREMENT

a. Observation made in the home cage.

b. Observation made in the open field.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Crl:CD(TM)BR VAF/Plus(TM) RATS

TABLE 4 (PAGE 1): FUNCTIONAL OBSERVATIONAL BATTERY - INDIVIDUAL DATA - FEMALE RATS

PREDOSAGE EXAMINATION

RAT #	9172	9181	9185	9196	9176	9189	9190	9197	9171	9180	9186	9191	9174	9177	9183	9187
DOSAGE GROUP	I	I	I	I	II	II	II	II	III	III	III	III	IV	IV	IV	IV
HOME CAGE BEHAVIOR	3	1	2	2	3	1	2	2	3	1	1	1	2	3	2	1
BEHAVIORAL OR POSTURAL ALTERATIONS a	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
REACTION TO REMOVAL	1	2	2	1	1	1	3	2	2	1	1	1	1	1	1	1
REACTION TO HANDLING	1	3	2	1	1	2	3	1	1	1	1	1	1	1	1	1
REARS	16	4	2	6	2	2	4	0	14	3	2	2	9	2	7	8
FECAL BOLUSES	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
URINE POOLS	0	4	0	0	0	2	2	1	0	0	1	0	0	1	0	1
LEVEL OF AROUSAL	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
BEHAVIORAL OR POSTURAL ALTERATIONS b	1	1	5	1	1	1	1	1	1	1	1	1	1	1	1	1
GAIT PATTERN	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SEVERITY OF GAIT ABNORMALITY	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
PALPEBRAL CLOSURE	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
PROMINENCE OF THE EYE	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
LACRIMATION	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SALIVATION	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
PILOBRECTION	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RESPIRATION	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
APPEARANCE	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
REACTION TO VISUAL STIMULUS	2	1	2	2	2	2	1	1	2	1	1	1	2	2	2	2
REACTION TO TACTILE STIMULUS	1	2	3	2	2	2	3	2	3	2	1	3	2	2	1	2
REACTION TO AUDITORY STIMULUS	3	3	3	3	3	3	3	2	3	3	3	2	3	3	3	2
REACTION TO TAIL PINCH	1	3	2	4	2	2	2	2	2	1	3	2	3	4	2	2
VISUAL PLACING RESPONSE	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1
AIR RIGHTING RESPONSE	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1
PUPILLARY RESPONSE	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
FORELIMB GRIP STRENGTH (G) #1	425	450	600	800	750	675	700	800	700	575	600	750	500	825	475	475
FORELIMB GRIP STRENGTH (G) #2	500	450	625	675	425	350	625	1100	425	500	625	1075	475	800	300	625
HINDLIMB GRIP STRENGTH (G) #1	650	625	300	450	300	575	700	500	575	620	475	425	475	575	475	325
HINDLIMB GRIP STRENGTH (G) #2	675	300	575	450	450	500	675	500	525	475	500	450	350	775	700	400
LANDING FOOT SPREAD (CM) #1	6.8	11.0	6.5	7.0	5.1	6.6	10.0	8.0	9.0	6.5	12.0	12.0	4.5	4.4	8.0	7.0
LANDING FOOT SPREAD (CM) #2	6.7	9.0	5.0	8.7	6.5	6.2	8.0	7.4	7.5	7.0	8.0	11.0	5.5	6.0	5.0	6.0
BODY WEIGHT	219	290	333	325	262	369	276	315	296	270	275	321	329	263	282	286

#1 - FIRST MEASUREMENT

#2 - SECOND MEASUREMENT

a. Observation made in the home cage.

b. Observation made in the open field.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 3 (PAGE 1): FUNCTIONAL OBSERVATIONAL BATTERY - INDIVIDUAL DATA - MALE RATS

TEST SESSION 2a

RAT #	19761	19762	19782	19787	19775	19780	19784	19788
DOSAGE GROUP b	I	I	I	I	IV	IV	IV	IV
HOME CAGE BEHAVIOR	3	2	2	2	1	1	1	1
BEHAVIORAL OR POSTURAL ALTERATIONS c	1	1	1	1	1	1	1	1
REACTION TO REMOVAL	1	1	1	1	1	1	1	1
REACTION TO HANDLING	1	1	1	1	1	1	1	2
REARS	4	3	3	0	2	3	5	4
FECAL BOLUSES	0	1	3	0	0	2	0	0
URINE POOLS	2	0	5	3	0	3	2	2
LEVEL OF AROUSAL	3	3	3	3	3	3	3	2
BEHAVIORAL OR POSTURAL ALTERATIONS d	1	1	1	1	2	1	2	2
GAIT PATTERN	1	1	1	1	1	1	1	2
SEVERITY OF GAIT ABNORMALITY	1	1	1	1	1	1	1	3
PALPEBRAL CLOSURE	1	1	1	1	1	1	1	1
PROMINENCE OF THE EYE	1	1	1	1	1	1	1	1
LACRIMATION	1	1	1	1	1	1	1	1
SALIVATION	1	1	1	1	1	1	1	1
PILOERECTION	0	0	0	0	0	0	0	0
RESPIRATION	0	0	0	0	0	0	0	0
APPEARANCE	1	1	1	1	1	1	1	1
REACTION TO VISUAL STIMULUS	2	1	2	2	2	1	1	2
REACTION TO TACTILE STIMULUS	2	1	2	2	2	1	1	2
REACTION TO AUDITORY STIMULUS	3	3	2	3	1	2	3	1
REACTION TO TAIL PINCH	2	2	2	4	2	2	2	4
VISUAL PLACING RESPONSE	1	1	1	1	1	1	1	1
AIR RIGHTING RESPONSE	1	1	1	1	2	1	2	1
PUPILLARY RESPONSE	1	1	1	1	0e	1	1	1
FORELIMB GRIP STRENGTH (G) #1	1325	825	1025	1050	350	750	1175	1050
FORELIMB GRIP STRENGTH (G) #2	950	850	875	575	325	850	875	850
HINDLIMB GRIP STRENGTH (G) #1	700	1000	500	400	450	775	650	450
HINDLIMB GRIP STRENGTH (G) #2	725	1000	525	775	325	925	700	600
LANDING FOOT SPLAY (CM) #1	7.7	8.5	8.1	10.0	6.6	5.4	11.5	15.0
LANDING FOOT SPLAY (CM) #2	6.1	8.6	6.0	9.7	7.7	7.5	9.5	10.5
BODY WEIGHT	495	471	468	361	290	408	345	415

#1 - FIRST MEASUREMENT

#2 - SECOND MEASUREMENT

- The examination was conducted before dosage on day 7 of dosage in Group I and day 4 postdosage in Group IV.
- Dosage occurred once daily for nine days in Group I and once daily for three days in Group IV.
- Observation made in the home cage.
- Observation made in the open field.
- Miosis identified.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 4 (PAGE 1): FUNCTIONAL OBSERVATIONAL BATTERY - INDIVIDUAL DATA - FEMALE RATS

TEST SESSION 2a

RAT #	9172	9181	9185	9196	9174	9177	9183	9187
DOSAGE GROUP b	I	I	I	I	IV	IV	IV	IV
HOME CAGE BEHAVIOR	3	1	2	1	2	1	1	1
BEHAVIORAL OR POSTURAL ALTERATIONS c	1	1	1	1	1	1	1	1
REACTION TO REMOVAL	1	1	1	1	1	1	1	1
REACTION TO HANDLING	1	1	1	1	1	1	1	1
REARS	15	7	0	10	8	2	0	2
FECAL BOLUSES	0	0	2	0	0	0	0	0
URINE POOLS	2	2	1	0	2	1	0	0
LEVEL OF AROUSAL	3	3	2	3	3	3	3	3
BEHAVIORAL OR POSTURAL ALTERATIONS d	1	1	5	1	1	1	2	1
GAIT PATTERN	1	1	1	1	1	1	2	1
SEVERITY OF GAIT ABNORMALITY	1	1	1	1	1	1	3	1
PALPEBRAL CLOSURE	1	1	1	1	1	1	1	1
PROMINENCE OF THE EYE	1	1	1	1	1	1	1	1
LACRIMATION	1	1	1	1	1	1	1	1
SALIVATION	1	1	1	1	1	1	1	1
PILOERECTION	0	0	0	0	0	0	0	0
RESPIRATION	0	0	0	0	0	0	0	0
APPEARANCE	1	1	1	1	1	1	1	1
REACTION TO VISUAL STIMULUS	2	2	2	1	2	2	2	2
REACTION TO TACTILE STIMULUS	2	2	2	1	2	2	1	1
REACTION TO AUDITORY STIMULUS	3	3	3	3	3	3	1	3
REACTION TO TAIL PINCH	2	2	2	1	4	2	1	2
VISUAL PLACING RESPONSE	1	1	1	1	1	1	1	1
AIR RIGHTING RESPONSE	2	1	1	1	1	1	2	1
PUPILLARY RESPONSE	1	1	1	1	1	1	1	1
FORELIMB GRIP STRENGTH (G) #1	650	575	1225	550	1075	625	350	600
FORELIMB GRIP STRENGTH (G) #2	275	475	600	425	675	525	200	425
HINDLIMB GRIP STRENGTH (G) #1	525	575	675	425	275	600	725	675
HINDLIMB GRIP STRENGTH (G) #2	475	475	700	475	575	800	775	450
LANDING FOOT SPLAY (CM) #1	6.2	8.0	6.0	6.7	6.5	5.3	9.6	7.5
LANDING FOOT SPLAY (CM) #2	7.0	6.5	5.8	6.3	6.0	5.0	11.0	8.5
BODY WEIGHT	223	290	338	305	282	254	226	279

#1 = FIRST MEASUREMENT

#2 = SECOND MEASUREMENT

- The examination was conducted before dosage on day 7 of dosage in Group I and day 4 postdosage in Group IV.
- Dosage occurred once daily for nine days in Group I and once daily for three days in Group IV.
- Observation made in the home cage.
- Observation made in the open field.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 3 (PAGE 2): FUNCTIONAL OBSERVATIONAL BATTERY - INDIVIDUAL DATA - MALE RATS

TEST SESSION 3a

RAT #	19761	19762	19782	19787	19775	19780	19784	19788
DOSAGE GROUP b	I	I	I	I	IV	IV	IV	IV
HOME CAGE BEHAVIOR	1	1	3	2	1	3	1	1
BEHAVIORAL OR POSTURAL ALTERATIONS c	1	1	1	1	1	1	1	1
REACTION TO REMOVAL	3	1	1	1	1	1	1	1
REACTION TO HANDLING	1	1	1	1	1	1	1	1
REARS	6	2	4	2	3	0	1	4
FECAL BOLUSES	0	0	0	0	3	4	0	2
URINE POOLS	2	0	5	2	3	2	1	4
LEVEL OF AROUSAL	3	3	3	3	3	3	2	3
BEHAVIORAL OR POSTURAL ALTERATIONS d	1	1	1	1	2	1	1	2
GAIT PATTERN	1	1	1	1	2	1	1	1
SEVERITY OF GAIT ABNORMALITY	1	1	1	1	2	1	1	1
PALPEBRAL CLOSURE	1	1	1	1	1	1	2	1
PROMINENCE OF THE EYE	1	1	1	1	1	1	1	1
LACRIMATION	1	1	1	1	1	1	1	1
SALIVATION	1	1	1	1	1	1	1	1
PILOBRECTION	0	0	0	0	0	0	0	0
RESPIRATION	0	0	0	0	0	0	1	0
APPEARANCE	1	1	1	1	1	1	1	1
REACTION TO VISUAL STIMULUS	2	2	2	2	2	2	1	2
REACTION TO TACTILE STIMULUS	1	2	2	2	1	2	1	2
REACTION TO AUDITORY STIMULUS	2	2	2	3	1	3	3	1
REACTION TO TAIL PINCH	2	1	4	4	1	3	2	2
VISUAL PLACING RESPONSE	1	1	1	1	2	1	1	1
AIR RIGHTING RESPONSE	1	1	1	1	1	2	1	3
PUPILLARY RESPONSE	1	1	1	1	0e	1	1	1
FORELIMB GRIP STRENGTH (G) #1	975	1000	850	800	350	750	425	900
FORELIMB GRIP STRENGTH (G) #2	975	1050	650	575	325	725	350	575
HINDLIMB GRIP STRENGTH (G) #1	600	750	450	575	450	800	550	500
HINDLIMB GRIP STRENGTH (G) #2	650	1350	475	825	325	975	500	450
LANDING FOOT SPREAD (CM) #1	5.3	9.2	5.6	5.0	6.6	7.9	5.5	16.0
LANDING FOOT SPREAD (CM) #2	4.8	8.1	5.5	6.6	7.7	9.8	7.4	10.5
BODY WEIGHT	519	479	477	374	290	438	316	461

#1 = FIRST MEASUREMENT

#2 = SECOND MEASUREMENT

a. The examination was conducted before dosage on day 4 postdosage in Group I and day 10 postdosage in Group IV.

b. Dosage occurred once daily for nine days in Group I and once daily for three days in Group IV.

c. Observation made in the home cage.

d. Observation made in the open field.

e. Miosis identified.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 4 (PAGE 2): FUNCTIONAL OBSERVATIONAL BATTERY - INDIVIDUAL DATA - FEMALE RATS

TEST SESSION 3a

RAT #	9172	9181	9185	9196	9174	9177	9183	9187
DOSAGE GROUP b	I	I	I	I	IV	IV	IV	IV
HOME CAGE BEHAVIOR	3	1	2	2	2	1	2	1
BEHAVIORAL OR POSTURAL ALTERATIONS c	1	1	1	1	1	1	2	1
REACTION TO REMOVAL	1	1	2	1	1	1	1	1
REACTION TO HANDLING	1	1	1	1	1	1	1	1
REARS	14	6	0	9	8	1	3	1
FECAL BOLUSES	0	0	0	0	0	1	0	4
URINE POOLS	0	1	0	0	2	0	1	1
LEVEL OF AROUSAL	3	3	2	3	3	3	3	3
BEHAVIORAL OR POSTURAL ALTERATIONS d	1	1	1	1	1	1	2,3	1
GAIT PATTERN	1	1	1	1	1	1	1	1
SEVERITY OF GAIT ABNORMALITY	1	1	1	1	1	1	1	1
PALPEBRAL CLOSURE	1	1	1	1	1	1	1	1
PROMINENCE OF THE EYE	1	1	1	1	1	1	1	1
LACRIMATION	1	1	1	1	1	1	1	1
SALIVATION	1	1	1	1	1	1	1	1
PILOERECTION	0	0	0	0	0	0	0	0
RESPIRATION	0	0	1	0	0	0	0	0
APPEARANCE	1	1	1	1	1	1	1	1
REACTION TO VISUAL STIMULUS	2	2	2	1	2	2	2	2
REACTION TO TACTILE STIMULUS	2	2	1	2	1	2	2	2
REACTION TO AUDITORY STIMULUS	3	3	3	1	3	3	2	2
REACTION TO TAIL PINCH	2	3	2	1	4	2	2	2
VISUAL PLACING RESPONSE	1	1	1	1	1	1	1	1
AIR RIGHTING RESPONSE	1	1	1	2	1	1	2	1
PUPILLARY RESPONSE	1	1	1	1	1	1	1	1
FORELIMB GRIP STRENGTH (G) #1	575	550	950	475	750	525	275	500
FORELIMB GRIP STRENGTH (G) #2	300	475	950	575	675	375	275	525
HINDLIMB GRIP STRENGTH (G) #1	425	400	650	375	500	750	625	400
HINDLIMB GRIP STRENGTH (G) #2	575	575	700	450	500	625	500	550
LANDING FOOT SPREAD (CM) #1	4.6	7.0	7.0	5.7	6.8	4.8	6.4	4.5
LANDING FOOT SPREAD (CM) #2	8.5	6.5	5.5	5.7	6.3	4.9	10.6	5.0
BODY WEIGHT	234	285	343	328	299	266	224	284

#1 - FIRST MEASUREMENT

#2 - SECOND MEASUREMENT

a. The examination was conducted before dosage on day 4 postdosage in Group I and day 10 postdosage in Group IV.

b. Dosage occurred once daily for nine days in Group I and once daily for three days in Group IV.

c. Observation made in the home cage.

d. Observation made in the open field.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Crl:CD(TM)BR VAF/Plus(TM) RATS

TABLE 5 (PAGE 1): MOTOR ACTIVITY - INDIVIDUAL DATA - MALE RATS - PREDOSAGE TEST

RAT # DOSAGE GROUP	19761 I	19762 I	19782 I	19787 I	19765 II	19767 II	19777 II	19785 II	19764 III	19768 III	19770 III	19776 III
NUMBER OF MOVEMENTS												
BLOCK 1	105	98	98	111	108	89	90	91	81	82	109	98
BLOCK 2	91	75	99	86	98	90	80	66	109	82	98	82
BLOCK 3	88	75	70	72	104	86	60	69	77	85	109	74
BLOCK 4	67	78	85	4	83	87	36	12	69	19	75	73
BLOCK 5	79	60	73	0	86	77	41	2	62	4	52	37
BLOCK 6	62	55	61	0	88	45	9	39	38	7	13	7
BLOCK 7	64	74	33	0	69	20	0	1	12	0	6	8
BLOCK 8	20	54	5	0	89	5	9	0	20	0	0	0
BLOCK 9	22	65	33	0	60	5	2	0	6	2	0	2
BLOCK 10	0	33	7	1	79	3	6	0	0	0	1	1
BLOCK 11	1	57	0	0	38	0	3	3	0	2	0	2
BLOCK 12	0	7	0	0	40	0	0	0	0	9	0	4
BLOCK 13	0	3	3	0	13	4	0	2	0	1	0	9
BLOCK 14	0	4	2	0	3	0	1	11	0	0	0	1
BLOCK 15	0	4	3	1	4	0	72	0	7	1	0	0
BLOCK 16	0	0	2	0	11	0	0	0	5	1	0	2
BLOCK 17	0	5	6	0	5	3	2	1	2	1	0	0
BLOCK 18	0	0	0	0	0	0	1	0	4	2	0	0
TOTAL	599	747	580	275	978	514	412	297	492	298	463	400

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.



PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 5 (PAGE 2): MOTOR ACTIVITY - INDIVIDUAL DATA - MALE RATS - PREDOSAGE TEST

RAT # DOSAGE GROUP	19761 I	19762 I	19782 I	19787 I	19765 II	19767 II	19777 II	19785 II	19764 III	19768 III	19770 III	19776 III
TIME (SECONDS) SPENT IN MOVEMENT												
BLOCK 1	256	240	242	252	261	231	205	231	254	273	245	234
BLOCK 2	207	171	249	212	246	221	178	121	255	255	244	204
BLOCK 3	202	172	140	171	214	213	132	147	181	240	242	182
BLOCK 4	127	201	190	9	204	222	76	24	169	37	142	162
BLOCK 5	170	132	194	0	198	192	71	1	132	6	159	57
BLOCK 6	131	136	143	0	216	92	16	78	85	14	23	14
BLOCK 7	132	166	67	0	142	34	0	0	21	0	14	10
BLOCK 8	26	138	5	0	179	7	19	0	31	0	0	0
BLOCK 9	48	139	66	0	174	6	2	0	12	3	0	3
BLOCK 10	0	82	12	1	190	3	15	0	0	0	1	2
BLOCK 11	2	134	0	0	83	0	3	6	0	2	0	4
BLOCK 12	0	15	0	0	75	0	0	0	0	8	0	5
BLOCK 13	0	4	5	0	27	9	0	1	0	1	0	15
BLOCK 14	0	6	1	3	0	0	1	23	0	0	0	0
BLOCK 15	0	5	2	0	5	0	149	0	14	0	0	0
BLOCK 16	0	0	2	0	15	0	0	0	5	1	0	9
BLOCK 17	0	9	17	0	4	10	3	1	2	2	0	0
BLOCK 18	0	0	0	0	0	0	0	0	5	2	0	0
TOTAL	1301	1750	1335	648	2233	1240	870	633	1166	844	1070	901

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 6 (PAGE 1): MOTOR ACTIVITY - INDIVIDUAL DATA - FEMALE RATS - PREDOSAGE TEST

RAT # DOSAGE GROUP	9172 I	9181 I	9185 I	9196 I	9176 II	9189 II	9190 II	9197 II	9171 III	9180 III	9186 III	9191 III
NUMBER OF MOVEMENTS												
BLOCK 1	88	83	72	101	85	90	65	91	88	95	92	70
BLOCK 2	74	46	83	101	107	63	53	89	74	84	57	59
BLOCK 3	48	7	5	82	85	57	20	29	51	95	34	80
BLOCK 4	46	7	0	56	37	64	6	55	33	59	6	55
BLOCK 5	74	1	1	72	0	57	2	32	31	23	2	58
BLOCK 6	63	5	7	76	3	58	0	5	4	25	0	30
BLOCK 7	48	1	11	28	15	34	2	0	4	14	2	65
BLOCK 8	19	4	4	59	0	28	0	1	0	2	0	27
BLOCK 9	49	4	2	25	0	1	0	0	0	55	2	1
BLOCK 10	39	1	14	63	0	0	1	0	0	87	0	1
BLOCK 11	10	1	11	10	33	1	0	4	0	39	0	0
BLOCK 12	27	2	1	24	0	2	0	0	18	15	6	0
BLOCK 13	50	1	1	18	1	0	9	1	6	4	2	0
BLOCK 14	3	1	2	18	2	4	0	2	0	0	5	0
BLOCK 15	0	43	1	16	0	0	0	0	0	1	2	0
BLOCK 16	4	4	18	0	21	3	5	1	0	0	0	0
BLOCK 17	1	0	5	0	55	0	0	2	1	1	4	1
BLOCK 18	1	0	1	0	8	2	1	1	1	3	0	0
TOTAL	644	211	239	749	452	464	164	313	311	602	214	447

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 6 (PAGE 2): MOTOR ACTIVITY - INDIVIDUAL DATA - FEMALE RATS - PREDOSAGE TEST

RAT # DOSAGE GROUP	9172 I	9181 I	9185 I	9196 I	9176 II	9189 II	9190 II	9197 II	9171 III	9180 III	9186 III	9191 III
TIME (SECONDS) SPENT IN MOVEMENT												
BLOCK 1	254	180	229	228	257	217	158	217	196	252	196	182
BLOCK 2	163	75	175	227	264	144	137	193	149	189	142	142
BLOCK 3	130	6	8	209	245	133	32	67	101	187	73	162
BLOCK 4	104	7	0	134	66	158	3	126	54	147	13	114
BLOCK 5	133	0	1	158	0	122	1	57	55	58	2	115
BLOCK 6	127	4	8	169	7	94	0	5	6	43	0	56
BLOCK 7	73	1	20	52	37	50	3	0	4	21	1	138
BLOCK 8	25	2	5	130	0	38	0	2	0	0	0	43
BLOCK 9	100	2	1	39	0	2	0	0	0	122	1	2
BLOCK 10	81	0	28	139	0	0	0	0	0	225	0	1
BLOCK 11	17	0	20	11	62	0	0	2	0	74	0	0
BLOCK 12	43	3	0	41	0	1	0	0	35	23	7	0
BLOCK 13	94	0	0	23	0	0	21	0	12	7	1	0
BLOCK 14	2	0	2	27	5	4	0	2	0	0	7	0
BLOCK 15	0	85	0	22	0	0	0	0	0	0	2	0
BLOCK 16	5	7	29	0	29	2	3	0	0	0	0	0
BLOCK 17	0	0	7	0	115	0	0	6	0	0	2	1
BLOCK 18	1	0	0	0	13	4	1	2	1	4	0	0
TOTAL	1352	372	533	1609	1100	969	359	679	613	1352	447	956

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Crl:CD(TM)BR VAF/Plus(TM) RATS

TABLE 3 (PAGE 1): FUNCTIONAL OBSERVATIONAL BATTERY - INDIVIDUAL DATA - MALE RATS

TEST SESSION 2a

RAT #	19761	19762	19782	19787	19764	19768	19770	19776
DOSAGE GROUP b	I	I	I	I	III	III	III	III
HOME CAGE BEHAVIOR	3	2	2	2	1	1	2	1
BEHAVIORAL OR POSTURAL ALTERATIONS c	1	1	1	1	1	1	1	1
REACTION TO REMOVAL	1	1	1	1	1	1	1	1
REACTION TO HANDLING	1	1	1	1	1	1	1	1
REARS	4	3	3	0	0	0	0	5
FECAL BOLUSES	0	1	3	0	0	1	0	0
URINE POOLS	2	0	5	3	1	2	1	0
LEVEL OF AROUSAL	3	3	3	3	3	3	2	3
BEHAVIORAL OR POSTURAL ALTERATIONS d	1	1	1	1	1	5	5	1
GAIT PATTERN	1	1	1	1	1	1	1	3
SEVERITY OF GAIT ABNORMALITY	1	1	1	1	1	1	1	2
PALPEBRAL CLOSURE	1	1	1	1	1	1	2	1
PROMINENCE OF THE EYE	1	1	1	1	1	1	1	1
LACRIMATION	1	1	1	1	1	1	1	1
SALIVATION	1	1	1	1	1	1	1	1
PILOERECTION	0	0	0	0	0	0	0	0
RESPIRATION	0	0	0	0	0	0	1	0
APPEARANCE	1	1	1	1	1	1	1	1
REACTION TO VISUAL STIMULUS	2	1	2	2	2	1	2	1
REACTION TO TACTILE STIMULUS	2	1	2	2	2	2	2	2
REACTION TO AUDITORY STIMULUS	3	3	2	3	3	3	3	2
REACTION TO TAIL PINCH	2	2	2	4	2	2	2	4
VISUAL PLACING RESPONSE	1	1	1	1	1	1	1	1
AIR RIGHTING RESPONSE	1	1	1	1	1	1	1	1
PUPILLARY RESPONSE	1	1	1	1	1	1	0	1
FORELIMB GRIP STRENGTH (G) #1	1325	825	1025	1050	825	425	900	1000
FORELIMB GRIP STRENGTH (G) #2	950	850	875	575	825	350	825	775
HINDLIMB GRIP STRENGTH (G) #1	700	1000	500	400	475	775	675	625
HINDLIMB GRIP STRENGTH (G) #2	725	1000	525	775	375	575	725	800
LANDING FOOT SPY (CM) #1	7.7	8.5	8.1	10.0	6.0	5.4	10.0	8.4
LANDING FOOT SPY (CM) #2	6.1	8.6	6.0	9.7	7.3	6.6	9.6	11.0
BODY WEIGHT	495	471	468	361	368	328	402	340

#1 - FIRST MEASUREMENT

#2 - SECOND MEASUREMENT

- The examination was conducted on day 7 of dosage, before dosage.
- Dosage occurred once daily for nine days.
- Observation made in the home cage.
- Observation made in the open field.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAP/Plus(TM) RATS

TABLE 3 (PAGE 2): FUNCTIONAL OBSERVATIONAL BATTERY - INDIVIDUAL DATA - MALE RATS

TEST SESSION 3a

RAT #	19761	19762	19782	19787	19764	19768	19770	19776
DOSAGE GROUP b	I	I	I	I	III	III	III	III
HOME CAGE BEHAVIOR	1	1	3	2	c	2	1	2
BEHAVIORAL OR POSTURAL ALTERATIONS d	1	1	1	1	c	1	1	1
REACTION TO REMOVAL	3	1	1	1	c	1	1	1
REACTION TO HANDLING	1	1	1	1	c	3	3	1
REARS	6	2	4	2	0	0	0	1
FECAL BOLUSES	0	0	0	0	4	1	0	0
URINE POOLS	2	0	5	2	3	c	0	0
LEVEL OF AROUSAL	3	3	3	3	2	2	2	3
BEHAVIORAL OR POSTURAL ALTERATIONS e	1	1	1	1	1	5	1	1
GAIT PATTERN	1	1	1	1	2	3	3	3
SEVERITY OF GAIT ABNORMALITY	1	1	1	1	3	3	4	3
PALPEBRAL CLOSURE	1	1	1	1	2	1	1	1
PROMINENCE OF THE EYE	1	1	1	1	1	1	1	1
LACRIMATION	1	1	1	1	1	1	1	1
SALIVATION	1	1	1	1	1	1	1	1
PILOERECTOR	0	0	0	0	0	0	0	0
RESPIRATION	0	0	0	0	0	1	1	0
APPEARANCE	1	1	1	1	1	1	1	1
REACTION TO VISUAL STIMULUS	2	2	2	2	2	1	1	1
REACTION TO TACTILE STIMULUS	1	2	2	2	1	2	1	1
REACTION TO AUDITORY STIMULUS	2	2	2	3	2	3	2	3
REACTION TO TAIL PINCH	2	1	4	4	2	4	1	2
VISUAL PLACING RESPONSE	1	1	1	1	1	1	2	1
AIR RIGHTING RESPONSE	1	1	1	1	2	1	1	2
PUPILLARY RESPONSE	1	1	1	1	1	0	1f	1
FORELIMB GRIP STRENGTH (G) #1	975	1000	850	800	625	425	325	875
FORELIMB GRIP STRENGTH (G) #2	975	1050	650	575	825	325	425	625
HINDLIMB GRIP STRENGTH (G) #1	600	750	450	575	450	625	400	1400
HINDLIMB GRIP STRENGTH (G) #2	650	1350	475	825	750	600	425	1350
LANDING FOOT SPREAD (CM) #1	5.3	9.2	5.6	5.0	12.0	6.3	11.2	13.6
LANDING FOOT SPREAD (CM) #2	4.8	8.1	5.5	6.6	13.5	11.8	12.8	12.7
BODY WEIGHT	519	479	477	374	417	367	349	374

#1 - FIRST MEASUREMENT

#2 - SECOND MEASUREMENT

- The examination was conducted on day 4 postdosage.
- Dosage occurred once daily for nine days.
- Value was not recorded.
- Observation made in the home cage.
- Observation made in the open field.
- Miosis identified.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 4 (PAGE 1): FUNCTIONAL OBSERVATIONAL BATTERY - INDIVIDUAL DATA - FEMALE RATS

TEST SESSION 2a

RAT #	9172	9181	9185	9196	9171	9180	9186	9191
DOSAGE GROUP b	I	I	I	I	III	III	III	III
HOME CAGE BEHAVIOR	3	1	2	1	1	1	1	1
BEHAVIORAL OR POSTURAL ALTERATIONS c	1	1	1	1	1	1	1	1
REACTION TO REMOVAL	1	1	1	1	3	1	1	3
REACTION TO HANDLING	1	1	1	1	4	4	1	4
REARS	15	7	0	10	0	0	0	0
FECAL BOLUSES	0	0	2	0	0	0	0	0
URINE POOLS	2	2	1	0	1	0	2	0
LEVEL OF AROUSAL	3	3	2	3	3	2	2	3
BEHAVIORAL OR POSTURAL ALTERATIONS d	1	1	5	1	1	1	5	5
GAIT PATTERN	1	1	1	1	3	1	3	3
SEVERITY OF GAIT ABNORMALITY	1	1	1	1	2	1	3	2
PALPEBRAL CLOSURE	1	1	1	1	1	2	1	2
PROMINENCE OF THE EYE	1	1	1	1	1	1	1	1
LACRIMATION	1	1	1	1	1	1	1	1
SALIVATION	1	1	1	1	1	1	1	1
PILOERECTION	0	0	0	0	0	0	0	0
RESPIRATION	0	0	0	0	0	0	0	0
APPEARANCE	1	1	1	1	1	1	1	1
REACTION TO VISUAL STIMULUS	2	2	2	1	2	1	2	1
REACTION TO TACTILE STIMULUS	2	2	2	1	2	2	1	2
REACTION TO AUDITORY STIMULUS	3	3	3	3	1	3	3	3
REACTION TO TAIL PINCH	2	2	2	1	3	2	2	2
VISUAL PLACING RESPONSE	1	1	1	1	1	1	1	1
AIR RIGHTING RESPONSE	2	1	1	1	1	1	1	1
PUPILLARY RESPONSE	1	1	1	1	1	1	1	1
FORELIMB GRIP STRENGTH (G) #1	650	575	1225	550	425	325	750	850
FORELIMB GRIP STRENGTH (G) #2	275	475	600	425	550	500	625	500
HINDLIMB GRIP STRENGTH (G) #1	525	575	675	425	375	450	525	300
HINDLIMB GRIP STRENGTH (G) #2	475	475	700	475	375	400	575	450
LANDING FOOT SPLAY (CM) #1	6.2	8.0	6.0	6.7	9.0	5.0	11.7	13.5
LANDING FOOT SPLAY (CM) #2	7.0	6.5	5.8	6.3	9.2	6.7	12.8	13.2
BODY WEIGHT	223	290	338	305	250	196	224	263

#1 - FIRST MEASUREMENT

#2 - SECOND MEASUREMENT

- a. The examination was conducted on day 7 of dosage, before dosage.
- b. Dosage occurred once daily for nine days.
- c. Observation made in the home cage.
- d. Observation made in the open field.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Crl:CD(TM)BR VAF/Plus(TM) RATS

TABLE 4 (PAGE 2): FUNCTIONAL OBSERVATIONAL BATTERY - INDIVIDUAL DATA - FEMALE RATS

TEST SESSION 3a

RAT #	9172	9181	9185	9196	9171	9180	9186	9191
DOSAGE GROUP b	I	I	I	I	III	III	III	III
HOME CAGE BEHAVIOR	3	1	2	2	1	2	1	1
BEHAVIORAL OR POSTURAL ALTERATIONS c	1	1	1	1	1	1	1	1
REACTION TO REMOVAL	1	1	2	1	1	1	1	1
REACTION TO HANDLING	1	1	1	1	2	1	1	1
REARS	14	6	0	9	0	0	0	0
FECAL BOLUSES	0	0	0	0	4	0	2	0
URINE POOLS	0	1	0	0	1	0	1	0
LEVEL OF AROUSAL	3	3	2	3	3	3	2	2
BEHAVIORAL OR POSTURAL ALTERATIONS d	1	1	1	1	1	1	1	1
GAIT PATTERN	1	1	1	1	3	2	3	3
SEVERITY OF GAIT ABNORMALITY	1	1	1	1	3	3	4	4
PALPEBRAL CLOSURE	1	1	1	1	2	1	2	3
PROMINENCE OF THE EYE	1	1	1	1	1	1	1	1
LACRIMATION	1	1	1	1	1	1	1	1
SALIVATION	1	1	1	1	1	1	1	1
PILOERECTOR	0	0	0	0	0	0	0	0
RESPIRATION	0	0	1	0	0	1	1	1
APPEARANCE	1	1	1	1	1	1	1	1
REACTION TO VISUAL STIMULUS	2	2	2	1	1	1	1	1
REACTION TO TACTILE STIMULUS	2	2	1	2	2	1	2	2
REACTION TO AUDITORY STIMULUS	3	3	3	1	3	3	3	3
REACTION TO TAIL PINCH	2	3	2	1	2	4	2	2
VISUAL PLACING RESPONSE	1	1	1	1	2	1	1	1
AIR RIGHTING RESPONSE	1	1	1	2	1	1	2	2
PUPILLARY RESPONSE	1	1	1	1	1	1	0e	1
FORELIMB GRIP STRENGTH (G) #1	575	550	950	475	400	225	625	300
FORELIMB GRIP STRENGTH (G) #2	300	475	950	575	425	300	575	525
HINDLIMB GRIP STRENGTH (G) #1	425	400	650	375	300	150	500	225
HINDLIMB GRIP STRENGTH (G) #2	575	575	700	450	450	350	500	250
LANDING FOOT SPREAD (CM) #1	4.6	7.0	7.0	5.7	12.4	9.5	13.0	9.5
LANDING FOOT SPREAD (CM) #2	8.5	6.5	5.5	5.7	11.0	11.4	12.5	13.7
BODY WEIGHT	234	285	343	328	286	195	248	264

#1 - FIRST MEASUREMENT

#2 - SECOND MEASUREMENT

- The examination was conducted on day 4 postdosage.
- Dosage occurred once daily for nine days.
- Observation made in the home cage.
- Observation made in the open field.
- Miosis identified.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Crl:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 1): MOTOR ACTIVITY - SUMMARY - PREDOSAGE TEST

GROUP		FEMALES	MALES
NUMBER OF RATS	N	28	28
NUMBER OF MOVEMENTS			
BLOCK 1	MEAN ± S.D.	84.3 ± 9.6	94.0 ± 10.1
BLOCK 2	MEAN ± S.D.	77.8 ± 15.2	82.4 ± 14.4
BLOCK 3	MEAN ± S.D.	56.2 ± 23.7	73.4 ± 19.9
BLOCK 4	MEAN ± S.D.	38.8 ± 22.7	56.6 ± 28.6
BLOCK 5	MEAN ± S.D.	32.2 ± 29.7	41.1 ± 30.0
BLOCK 6	MEAN ± S.D.	23.2 ± 26.0	32.1 ± 26.2
BLOCK 7	MEAN ± S.D.	15.6 ± 21.4	21.7 ± 25.8
BLOCK 8	MEAN ± S.D.	11.1 ± 18.3	13.2 ± 21.8
BLOCK 9	MEAN ± S.D.	9.4 ± 18.0	11.1 ± 20.5
BLOCK 10	MEAN ± S.D.	11.1 ± 21.6	8.7 ± 21.8
BLOCK 11	MEAN ± S.D.	4.3 ± 9.5	5.2 ± 13.1
BLOCK 12	MEAN ± S.D.	3.9 ± 7.5	4.4 ± 9.5
BLOCK 13	MEAN ± S.D.	4.9 ± 10.9	3.1 ± 7.0
BLOCK 14	MEAN ± S.D.	4.8 ± 13.1	3.5 ± 7.3
BLOCK 15	MEAN ± S.D.	2.8 ± 8.6	6.8 ± 17.3
BLOCK 16	MEAN ± S.D.	3.5 ± 6.9	2.1 ± 5.0
BLOCK 17	MEAN ± S.D.	5.4 ± 15.6	1.6 ± 2.7
BLOCK 18	MEAN ± S.D.	2.3 ± 4.6	2.5 ± 9.4
TOTAL	MEAN ± S.D.	391.5 ± 148.3	463.5 ± 177.0

STATISTICAL ANALYSES OF MOTOR ACTIVITY WERE RESTRICTED TO THE TOTAL NUMBER OF MOVEMENTS AND TOTAL TIME SPENT IN MOVEMENT.  
TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.



PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 2): MOTOR ACTIVITY - SUMMARY - PREDOSAGE TEST

GROUP		FEMALES	MALES
NUMBER OF RATS	N	28	28
TIME (SECONDS) SPENT IN MOVEMENT			
BLOCK 1	MEAN ± S.D.	211.4 ± 28.9	227.1 ± 29.4
BLOCK 2	MEAN ± S.D.	174.2 ± 40.7	198.2 ± 42.3
BLOCK 3	MEAN ± S.D.	125.7 ± 59.6	169.1 ± 50.0
BLOCK 4	MEAN ± S.D.	82.1 ± 54.5	126.9 ± 66.7
BLOCK 5	MEAN ± S.D.	66.6 ± 68.8	88.1 ± 70.5
BLOCK 6	MEAN ± S.D.	46.6 ± 57.1	69.7 ± 60.6
BLOCK 7	MEAN ± S.D.	30.8 ± 46.6	43.9 ± 54.8
BLOCK 8	MEAN ± S.D.	20.5 ± 39.2	25.8 ± 46.2
BLOCK 9	MEAN ± S.D.	17.3 ± 35.9	24.8 ± 48.6
BLOCK 10	MEAN ± S.D.	23.0 ± 51.4	19.6 ± 51.5
BLOCK 11	MEAN ± S.D.	7.1 ± 18.0	10.6 ± 29.7
BLOCK 12	MEAN ± S.D.	6.1 ± 12.8	7.5 ± 18.0
BLOCK 13	MEAN ± S.D.	8.8 ± 21.4	5.2 ± 13.3
BLOCK 14	MEAN ± S.D.	8.1 ± 27.9	5.6 ± 13.9
BLOCK 15	MEAN ± S.D.	5.1 ± 16.8	14.7 ± 44.8
BLOCK 16	MEAN ± S.D.	5.0 ± 11.0	3.6 ± 11.9
BLOCK 17	MEAN ± S.D.	9.6 ± 30.4	3.3 ± 6.3
BLOCK 18	MEAN ± S.D.	4.0 ± 8.8	4.5 ± 19.6
TOTAL	MEAN ± S.D.	851.9 ± 331.7	1048.4 ± 402.0

STATISTICAL ANALYSES OF MOTOR ACTIVITY WERE RESTRICTED TO THE TOTAL NUMBER OF MOVEMENTS AND TOTAL TIME SPENT IN MOVEMENT.  
TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A FIVE MINUTE PERIOD.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Crl:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 1): MOTOR ACTIVITY - SUMMARY

DOSAGE GROUP		I	III
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, SALINE)	40(ACRYLAMIDE)
<u>TEST SESSION 2b</u>			
NUMBER OF RATS	N	8	8
NUMBER OF MOVEMENTS			
BLOCK 1	MEAN ± S.D.	86.2 ± 9.3	53.4 ± 24.4
BLOCK 2	MEAN ± S.D.	76.0 ± 7.6	49.4 ± 21.9
BLOCK 3	MEAN ± S.D.	50.4 ± 21.5	23.8 ± 25.6
BLOCK 4	MEAN ± S.D.	34.8 ± 28.3	13.0 ± 17.4
BLOCK 5	MEAN ± S.D.	25.1 ± 23.8	10.1 ± 11.9
BLOCK 6	MEAN ± S.D.	15.0 ± 21.8	4.8 ± 4.0
BLOCK 7	MEAN ± S.D.	13.0 ± 26.9	8.0 ± 11.4
BLOCK 8	MEAN ± S.D.	8.9 ± 25.1	5.0 ± 3.7
BLOCK 9	MEAN ± S.D.	5.6 ± 15.5	11.5 ± 21.9
BLOCK 10	MEAN ± S.D.	11.1 ± 31.1	3.0 ± 2.0
BLOCK 11	MEAN ± S.D.	8.0 ± 21.4	2.9 ± 2.3
BLOCK 12	MEAN ± S.D.	7.4 ± 17.7	3.4 ± 4.6
BLOCK 13	MEAN ± S.D.	7.1 ± 17.0	3.6 ± 3.0
BLOCK 14	MEAN ± S.D.	7.1 ± 18.2	4.2 ± 4.1
BLOCK 15	MEAN ± S.D.	1.5 ± 2.2	2.5 ± 2.3
BLOCK 16	MEAN ± S.D.	1.4 ± 1.4	5.6 ± 7.1
BLOCK 17	MEAN ± S.D.	0.2 ± 0.7	4.6 ± 6.6
BLOCK 18	MEAN ± S.D.	0.9 ± 1.2	6.4 ± 7.6
TOTAL	MEAN ± S.D.	359.8 ± 226.4	215.1 ± 72.5

STATISTICAL ANALYSES OF MOTOR ACTIVITY WERE RESTRICTED TO THE TOTAL NUMBER OF MOVEMENTS AND TOTAL TIME SPENT IN MOVEMENT.  
TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

- Dosage occurred once daily for nine days.
- The test was conducted on day 7 of dosage, before dosage and after completion of the functional observational battery.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 2): MOTOR ACTIVITY - SUMMARY

DOSAGE GROUP		I		III	
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, SALINE)		40(ACRYLAMIDE)	
<u>TEST SESSION 2b</u>					
NUMBER OF RATS	N	8		8	
TIME (SECONDS) SPENT IN MOVEMENT					
BLOCK 1	MEAN ± S.D.	206.5 ±	27.8	130.8 ±	62.9
BLOCK 2	MEAN ± S.D.	172.5 ±	29.6	113.6 ±	54.4
BLOCK 3	MEAN ± S.D.	106.1 ±	57.6	54.4 ±	67.6
BLOCK 4	MEAN ± S.D.	74.4 ±	65.5	32.1 ±	50.0
BLOCK 5	MEAN ± S.D.	49.5 ±	48.6	18.8 ±	26.3
BLOCK 6	MEAN ± S.D.	28.2 ±	46.6	5.8 ±	6.0
BLOCK 7	MEAN ± S.D.	27.0 ±	61.6	13.6 ±	24.8
BLOCK 8	MEAN ± S.D.	19.8 ±	55.9	7.6 ±	6.0
BLOCK 9	MEAN ± S.D.	10.8 ±	30.4	22.9 ±	50.9
BLOCK 10	MEAN ± S.D.	20.9 ±	58.2	5.2 ±	3.6
BLOCK 11	MEAN ± S.D.	18.1 ±	49.3	4.8 ±	3.3
BLOCK 12	MEAN ± S.D.	16.8 ±	42.2	4.0 ±	5.9
BLOCK 13	MEAN ± S.D.	12.9 ±	32.9	4.0 ±	3.1
BLOCK 14	MEAN ± S.D.	11.8 ±	31.6	5.9 ±	4.9
BLOCK 15	MEAN ± S.D.	2.0 ±	3.2	4.9 ±	3.8
BLOCK 16	MEAN ± S.D.	2.5 ±	3.5	8.2 ±	17.0
BLOCK 17	MEAN ± S.D.	0.0 ±	0.0	11.9 ±	21.0
BLOCK 18	MEAN ± S.D.	0.4 ±	0.7	14.0 ±	20.8
TOTAL	MEAN ± S.D.	780.0 ±	476.4	462.4 ±	188.1

STATISTICAL ANALYSES OF MOTOR ACTIVITY WERE RESTRICTED TO THE TOTAL NUMBER OF MOVEMENTS AND TOTAL TIME SPENT IN MOVEMENT.

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred once daily for nine days.

b. The test was conducted on day 7 of dosage, before dosage and after completion of the functional observational battery.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Crl:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 3): MOTOR ACTIVITY - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY) <sup>a</sup>		I 0(VEHICLE, SALINE)	III 40(ACRYLAMIDE)
<u>TEST SESSION 3b</u>			
NUMBER OF RATS	N	8	8
NUMBER OF MOVEMENTS			
BLOCK 1	MEAN ± S.D.	86.0 ± 5.7	46.4 ± 20.3
BLOCK 2	MEAN ± S.D.	68.6 ± 25.5	35.6 ± 20.9
BLOCK 3	MEAN ± S.D.	47.1 ± 31.5	23.0 ± 21.5
BLOCK 4	MEAN ± S.D.	43.2 ± 36.4	15.1 ± 18.3
BLOCK 5	MEAN ± S.D.	26.5 ± 28.3	8.9 ± 17.5
BLOCK 6	MEAN ± S.D.	27.0 ± 33.6	3.1 ± 3.6
BLOCK 7	MEAN ± S.D.	25.0 ± 32.8	1.4 ± 1.7
BLOCK 8	MEAN ± S.D.	18.4 ± 28.0	2.0 ± 3.4
BLOCK 9	MEAN ± S.D.	11.8 ± 18.0	3.2 ± 3.3
BLOCK 10	MEAN ± S.D.	7.9 ± 18.0	0.9 ± 0.8
BLOCK 11	MEAN ± S.D.	7.2 ± 12.5	1.6 ± 1.4
BLOCK 12	MEAN ± S.D.	0.0 ± 0.0	2.0 ± 2.8
BLOCK 13	MEAN ± S.D.	0.8 ± 1.2	1.8 ± 2.0
BLOCK 14	MEAN ± S.D.	1.1 ± 2.1	4.9 ± 4.2
BLOCK 15	MEAN ± S.D.	1.0 ± 2.4	0.5 ± 0.8
BLOCK 16	MEAN ± S.D.	0.1 ± 0.4	2.0 ± 2.1
BLOCK 17	MEAN ± S.D.	0.9 ± 1.4	4.1 ± 7.5
BLOCK 18	MEAN ± S.D.	1.9 ± 2.5	2.8 ± 3.1
TOTAL	MEAN ± S.D.	374.5 ± 204.3	159.2 ± 78.4*

STATISTICAL ANALYSES OF MOTOR ACTIVITY WERE RESTRICTED TO THE TOTAL NUMBER OF MOVEMENTS AND TOTAL TIME SPENT IN MOVEMENT.  
TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

- a. Dosage occurred once daily for nine days.
- b. The test was conducted on day 4 postdosage after completion of the functional observational battery.
- \* Significantly different from the vehicle control group TOTAL ( $P \leq 0.05$ ).

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 4): MOTOR ACTIVITY - SUMMARY

DOSAGE GROUP		I	III
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, SALINE)	40(ACRYLAMIDE)
<u>TEST SESSION 3b</u>			
NUMBER OF RATS	N	8	8
TIME (SECONDS) SPENT IN MOVEMENT			
BLOCK 1	MEAN ± S.D.	205.5 ± 19.2	115.9 ± 52.6
BLOCK 2	MEAN ± S.D.	154.9 ± 67.8	105.6 ± 73.6
BLOCK 3	MEAN ± S.D.	113.5 ± 80.4	58.0 ± 66.5
BLOCK 4	MEAN ± S.D.	98.1 ± 81.6	29.8 ± 43.5
BLOCK 5	MEAN ± S.D.	63.4 ± 69.4	21.0 ± 52.2
BLOCK 6	MEAN ± S.D.	58.6 ± 77.1	4.4 ± 5.3
BLOCK 7	MEAN ± S.D.	53.2 ± 69.5	2.1 ± 3.3
BLOCK 8	MEAN ± S.D.	37.8 ± 61.5	2.1 ± 3.5
BLOCK 9	MEAN ± S.D.	24.0 ± 42.6	7.0 ± 8.5
BLOCK 10	MEAN ± S.D.	14.9 ± 32.9	1.4 ± 1.7
BLOCK 11	MEAN ± S.D.	12.0 ± 21.1	2.5 ± 2.6
BLOCK 12	MEAN ± S.D.	0.0 ± 0.0	3.8 ± 5.8
BLOCK 13	MEAN ± S.D.	0.5 ± 1.1	3.0 ± 3.8
BLOCK 14	MEAN ± S.D.	1.0 ± 1.8	7.0 ± 7.5
BLOCK 15	MEAN ± S.D.	2.4 ± 5.6	0.4 ± 0.5
BLOCK 16	MEAN ± S.D.	0.1 ± 0.4	2.0 ± 3.5
BLOCK 17	MEAN ± S.D.	1.0 ± 1.4	8.0 ± 18.4
BLOCK 18	MEAN ± S.D.	2.4 ± 2.9	5.8 ± 7.1
TOTAL	MEAN ± S.D.	843.2 ± 463.3	379.6 ± 238.2*

STATISTICAL ANALYSES OF MOTOR ACTIVITY WERE RESTRICTED TO THE TOTAL NUMBER OF MOVEMENTS AND TOTAL TIME SPENT IN MOVEMENT.  
TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

- a. Dosage occurred once daily for nine days.
- b. The test was conducted on day 4 postdosage after completion of the functional observational battery.
- \* Significantly different from the vehicle control group TOTAL ( $P \leq 0.05$ ).

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 1): MOTOR ACTIVITY - SUMMARY

DOSAGE GROUP		I	IV
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, SALINE)	200(IDPN)
<u>TEST SESSION 2b</u>			
NUMBER OF RATS	N	8	8
NUMBER OF MOVEMENTS			
BLOCK 1	MEAN ± S.D.	86.2 ± 9.3	82.6 ± 12.7
BLOCK 2	MEAN ± S.D.	76.0 ± 7.6	45.8 ± 28.9
BLOCK 3	MEAN ± S.D.	50.4 ± 21.5	29.2 ± 31.6
BLOCK 4	MEAN ± S.D.	34.8 ± 28.3	23.0 ± 31.5
BLOCK 5	MEAN ± S.D.	25.1 ± 23.8	15.4 ± 21.0
BLOCK 6	MEAN ± S.D.	15.0 ± 21.8	7.4 ± 8.9
BLOCK 7	MEAN ± S.D.	13.0 ± 26.9	11.8 ± 20.0
BLOCK 8	MEAN ± S.D.	8.9 ± 25.1	8.2 ± 12.4
BLOCK 9	MEAN ± S.D.	5.6 ± 15.5	1.4 ± 2.1
BLOCK 10	MEAN ± S.D.	11.1 ± 31.1	0.2 ± 0.7
BLOCK 11	MEAN ± S.D.	8.0 ± 21.4	2.8 ± 3.5
BLOCK 12	MEAN ± S.D.	7.4 ± 17.7	14.1 ± 21.6
BLOCK 13	MEAN ± S.D.	7.1 ± 17.0	13.0 ± 23.3
BLOCK 14	MEAN ± S.D.	7.1 ± 18.2	4.6 ± 11.9
BLOCK 15	MEAN ± S.D.	1.5 ± 2.2	0.8 ± 2.1
BLOCK 16	MEAN ± S.D.	1.4 ± 1.4	6.9 ± 9.4
BLOCK 17	MEAN ± S.D.	0.2 ± 0.7	5.2 ± 14.0
BLOCK 18	MEAN ± S.D.	0.9 ± 1.2	5.2 ± 10.7
TOTAL	MEAN ± S.D.	359.8 ± 226.4	277.6 ± 123.4

STATISTICAL ANALYSES OF MOTOR ACTIVITY WERE RESTRICTED TO THE TOTAL NUMBER OF MOVEMENTS AND TOTAL TIME SPENT IN MOVEMENT.

TOTAL - SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

- Dosage occurred once daily for nine days in Group I and once daily for three days in Group IV.
- The test was conducted on day 7 of dosage in Group I and day 4 postdosage in Group IV, after completion of the functional observational battery.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Crl:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 2): MOTOR ACTIVITY - SUMMARY

DOSAGE GROUP	I	IV
DOSAGE (MG/KG/DAY) <sup>a</sup>	0(VEHICLE, SALINE)	200(IDPN)

TEST SESSION 2b

NUMBER OF RATS	N	8	8
TIME (SECONDS) SPENT IN MOVEMENT			
BLOCK 1	MEAN ± S.D.	206.5 ± 27.8	199.1 ± 37.9
BLOCK 2	MEAN ± S.D.	172.5 ± 29.6	108.6 ± 73.8
BLOCK 3	MEAN ± S.D.	106.1 ± 57.6	63.9 ± 75.7
BLOCK 4	MEAN ± S.D.	74.4 ± 65.5	47.8 ± 70.8
BLOCK 5	MEAN ± S.D.	49.5 ± 48.6	38.8 ± 54.8
BLOCK 6	MEAN ± S.D.	28.2 ± 46.6	15.8 ± 21.2
BLOCK 7	MEAN ± S.D.	27.0 ± 61.6	26.2 ± 50.4
BLOCK 8	MEAN ± S.D.	19.8 ± 55.9	15.4 ± 27.2
BLOCK 9	MEAN ± S.D.	10.8 ± 30.4	1.2 ± 2.5
BLOCK 10	MEAN ± S.D.	20.9 ± 58.2	0.2 ± 0.7
BLOCK 11	MEAN ± S.D.	18.1 ± 49.3	5.4 ± 8.2
BLOCK 12	MEAN ± S.D.	16.8 ± 42.2	26.8 ± 44.1
BLOCK 13	MEAN ± S.D.	12.9 ± 32.9	26.4 ± 46.8
BLOCK 14	MEAN ± S.D.	11.8 ± 31.6	8.0 ± 21.8
BLOCK 15	MEAN ± S.D.	2.0 ± 3.2	0.8 ± 2.1
BLOCK 16	MEAN ± S.D.	2.5 ± 3.5	12.5 ± 18.7
BLOCK 17	MEAN ± S.D.	0.0 ± 0.0	10.8 ± 28.8
BLOCK 18	MEAN ± S.D.	0.4 ± 0.7	9.1 ± 21.3
TOTAL	MEAN ± S.D.	780.0 ± 476.4	616.6 ± 277.5

STATISTICAL ANALYSES OF MOTOR ACTIVITY WERE RESTRICTED TO THE TOTAL NUMBER OF MOVEMENTS AND TOTAL TIME SPENT IN MOVEMENT.

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

- Dosage occurred once daily for nine days in Group I and once daily for three days in Group IV.
- The test was conducted on day 7 of dosage in Group I and day 4 postdosage in Group IV, after completion of the functional observational battery.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 3): MOTOR ACTIVITY - SUMMARY

DOSAGE GROUP		I	IV
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, SALINE)	200(IDPN)
<u>TEST SESSION 3b</u>			
NUMBER OF RATS	N	8	8
NUMBER OF MOVEMENTS			
BLOCK 1	MEAN ± S.D.	86.0 ± 5.7	85.8 ± 12.2
BLOCK 2	MEAN ± S.D.	68.6 ± 25.5	64.0 ± 19.2
BLOCK 3	MEAN ± S.D.	47.1 ± 31.5	37.5 ± 25.7
BLOCK 4	MEAN ± S.D.	43.2 ± 36.4	12.2 ± 18.8
BLOCK 5	MEAN ± S.D.	26.5 ± 28.3	10.9 ± 17.3
BLOCK 6	MEAN ± S.D.	27.0 ± 33.6	3.6 ± 6.8
BLOCK 7	MEAN ± S.D.	25.0 ± 32.8	2.0 ± 4.9
BLOCK 8	MEAN ± S.D.	18.4 ± 28.0	0.8 ± 1.0
BLOCK 9	MEAN ± S.D.	11.8 ± 18.0	4.8 ± 13.0
BLOCK 10	MEAN ± S.D.	7.9 ± 18.0	11.0 ± 31.1
BLOCK 11	MEAN ± S.D.	7.2 ± 12.5	11.5 ± 25.6
BLOCK 12	MEAN ± S.D.	0.0 ± 0.0	2.6 ± 3.4
BLOCK 13	MEAN ± S.D.	0.8 ± 1.2	0.6 ± 0.9
BLOCK 14	MEAN ± S.D.	1.1 ± 2.1	1.6 ± 2.4
BLOCK 15	MEAN ± S.D.	1.0 ± 2.4	0.2 ± 0.7
BLOCK 16	MEAN ± S.D.	0.1 ± 0.4	4.6 ± 8.5
BLOCK 17	MEAN ± S.D.	0.9 ± 1.4	10.6 ± 17.3
BLOCK 18	MEAN ± S.D.	1.9 ± 2.5	10.0 ± 20.8
TOTAL	MEAN ± S.D.	374.5 ± 204.3	274.4 ± 119.3

STATISTICAL ANALYSES OF MOTOR ACTIVITY WERE RESTRICTED TO THE TOTAL NUMBER OF MOVEMENTS AND TOTAL TIME SPENT IN MOVEMENT.

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

- Dosage occurred once daily for nine days in Group I and once daily for three days in Group IV.
- The test was conducted on day 4 postdosage in Group I and day 10 postdosage in Group IV, after completion of the functional observational battery.



PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Crl:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 4): MOTOR ACTIVITY - SUMMARY

DOSAGE GROUP		I	IV
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, SALINE)	200(IDPN)
<u>TEST SESSION 3b</u>			
NUMBER OF RATS	N	8	8
TIME (SECONDS) SPENT IN MOVEMENT			
BLOCK 1	MEAN ± S.D.	205.5 ± 19.2	214.2 ± 28.2
BLOCK 2	MEAN ± S.D.	154.9 ± 67.8	145.4 ± 46.7
BLOCK 3	MEAN ± S.D.	113.5 ± 80.4	85.6 ± 63.8
BLOCK 4	MEAN ± S.D.	98.1 ± 81.6	21.6 ± 30.8
BLOCK 5	MEAN ± S.D.	63.4 ± 69.4	23.8 ± 40.9
BLOCK 6	MEAN ± S.D.	58.6 ± 77.1	6.9 ± 17.5
BLOCK 7	MEAN ± S.D.	53.2 ± 69.5	2.4 ± 5.6
BLOCK 8	MEAN ± S.D.	37.8 ± 61.5	1.0 ± 1.2
BLOCK 9	MEAN ± S.D.	24.0 ± 42.6	11.9 ± 33.6
BLOCK 10	MEAN ± S.D.	14.9 ± 32.9	24.9 ± 70.4
BLOCK 11	MEAN ± S.D.	12.0 ± 21.1	24.4 ± 59.6
BLOCK 12	MEAN ± S.D.	0.0 ± 0.0	5.8 ± 7.1
BLOCK 13	MEAN ± S.D.	0.5 ± 1.1	0.9 ± 1.2
BLOCK 14	MEAN ± S.D.	1.0 ± 1.8	1.4 ± 2.2
BLOCK 15	MEAN ± S.D.	2.4 ± 5.6	0.2 ± 0.7
BLOCK 16	MEAN ± S.D.	0.1 ± 0.4	11.9 ± 21.7
BLOCK 17	MEAN ± S.D.	1.0 ± 1.4	25.6 ± 44.9
BLOCK 18	MEAN ± S.D.	2.4 ± 2.9	20.5 ± 45.7
TOTAL	MEAN ± S.D.	843.2 ± 463.3	628.2 ± 283.6

STATISTICAL ANALYSES OF MOTOR ACTIVITY WERE RESTRICTED TO THE TOTAL NUMBER OF MOVEMENTS AND TOTAL TIME SPENT IN MOVEMENT.

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred once daily for nine days in Group I and once daily for three days in Group IV.

b. The test was conducted on day 4 postdosage in Group I and day 10 postdosage in Group IV, after completion of the functional observational battery.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Crl:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 1): MOTOR ACTIVITY - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY) <sup>a</sup>		II 0(VEHICLE, CORN OIL)	V 75(CARBARYL)
TEST SESSION 2b			
NUMBER OF RATS	N	8	8
NUMBER OF MOVEMENTS			
BLOCK 1	MEAN ± S.D.	86.5 ± 11.7	17.8 ± 9.2
BLOCK 2	MEAN ± S.D.	82.5 ± 13.0	5.6 ± 6.1
BLOCK 3	MEAN ± S.D.	61.1 ± 19.4	6.2 ± 6.6
BLOCK 4	MEAN ± S.D.	51.1 ± 33.0	3.4 ± 4.2
BLOCK 5	MEAN ± S.D.	38.1 ± 27.8	2.6 ± 2.0
BLOCK 6	MEAN ± S.D.	21.6 ± 24.4	5.0 ± 5.1
BLOCK 7	MEAN ± S.D.	11.5 ± 10.9	8.4 ± 10.2
BLOCK 8	MEAN ± S.D.	14.0 ± 12.5	6.6 ± 6.7
BLOCK 9	MEAN ± S.D.	10.5 ± 13.5	5.8 ± 10.8
BLOCK 10	MEAN ± S.D.	2.4 ± 1.9	9.2 ± 8.8
BLOCK 11	MEAN ± S.D.	0.9 ± 0.8	7.1 ± 7.4
BLOCK 12	MEAN ± S.D.	1.2 ± 1.8	5.6 ± 6.1
BLOCK 13	MEAN ± S.D.	3.9 ± 10.2	5.2 ± 4.2
BLOCK 14	MEAN ± S.D.	1.5 ± 2.8	9.0 ± 12.9
BLOCK 15	MEAN ± S.D.	1.0 ± 1.4	5.2 ± 4.7
BLOCK 16	MEAN ± S.D.	1.9 ± 1.6	10.2 ± 13.5
BLOCK 17	MEAN ± S.D.	10.6 ± 23.0	5.6 ± 6.2
BLOCK 18	MEAN ± S.D.	6.6 ± 10.5	6.8 ± 9.8
TOTAL	MEAN ± S.D.	407.0 ± 99.0	125.5 ± 109.5**

STATISTICAL ANALYSES OF MOTOR ACTIVITY WERE RESTRICTED TO THE TOTAL NUMBER OF MOVEMENTS AND TOTAL TIME SPENT IN MOVEMENT.

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

- a. Dosage occurred once.
- b. The test was conducted approximately 5.5 hours postdosage in Group II and 1 hour postdosage in Group V, after completion of the functional observational battery.

\*\* Significantly different from the vehicle control group TOTAL ( $P \leq 0.01$ ).

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 2): MOTOR ACTIVITY - SUMMARY

DOSAGE GROUP		II	V
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, CORN OIL)	75(CARBARYL)
<u>TEST SESSION 2b</u>			
NUMBER OF RATS	N	6	8
TIME (SECONDS) SPENT IN MOVEMENT			
BLOCK 1	MEAN ± S.D.	205.0 ± 29.4	35.2 ± 23.7
BLOCK 2	MEAN ± S.D.	185.1 ± 40.7	8.4 ± 11.3
BLOCK 3	MEAN ± S.D.	140.6 ± 54.3	9.6 ± 14.1
BLOCK 4	MEAN ± S.D.	109.6 ± 79.2	2.8 ± 3.5
BLOCK 5	MEAN ± S.D.	77.2 ± 57.7	3.5 ± 6.1
BLOCK 6	MEAN ± S.D.	42.6 ± 52.8	8.6 ± 12.2
BLOCK 7	MEAN ± S.D.	25.2 ± 28.0	12.6 ± 16.2
BLOCK 8	MEAN ± S.D.	29.0 ± 33.1	10.4 ± 12.4
BLOCK 9	MEAN ± S.D.	20.9 ± 34.0	9.5 ± 22.1
BLOCK 10	MEAN ± S.D.	2.1 ± 3.2	12.4 ± 14.7
BLOCK 11	MEAN ± S.D.	0.8 ± 0.9	8.9 ± 11.0
BLOCK 12	MEAN ± S.D.	1.2 ± 2.2	9.8 ± 16.2
BLOCK 13	MEAN ± S.D.	6.8 ± 18.7	8.4 ± 8.1
BLOCK 14	MEAN ± S.D.	2.9 ± 5.8	16.2 ± 24.6
BLOCK 15	MEAN ± S.D.	1.9 ± 2.7	8.0 ± 10.3
BLOCK 16	MEAN ± S.D.	2.2 ± 2.1	14.1 ± 20.9
BLOCK 17	MEAN ± S.D.	19.4 ± 44.6	8.6 ± 13.2
BLOCK 18	MEAN ± S.D.	8.9 ± 14.4	9.9 ± 15.2
TOTAL	MEAN ± S.D.	881.5 ± 235.4	196.9 ± 205.8**

STATISTICAL ANALYSES OF MOTOR ACTIVITY WERE RESTRICTED TO THE TOTAL NUMBER OF MOVEMENTS AND TOTAL TIME SPENT IN MOVEMENT.

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred once.

b. The test was conducted approximately 5.5 hours postdosage in Group II and 1 hour postdosage in Group V, after completion of the functional observational battery.

\*\* Significantly different from the vehicle control group TOTAL ( $P \leq 0.01$ ).

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Crl:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 1): MOTOR ACTIVITY - SUMMARY

DOSAGE GROUP		II	VI
DOSAGE (MG/KG/DAY)a		0(VEHICLE, CORN OIL)	75(DDT)
<u>TEST SESSION 2b</u>			
NUMBER OF RATS	N	8	8
NUMBER OF MOVEMENTS			
BLOCK 1	MEAN ± S.D.	86.5 ± 11.7	71.1 ± 11.6
BLOCK 2	MEAN ± S.D.	82.5 ± 13.0	76.2 ± 10.3
BLOCK 3	MEAN ± S.D.	61.1 ± 19.4	56.9 ± 17.8
BLOCK 4	MEAN ± S.D.	51.1 ± 23.0	41.9 ± 19.0
BLOCK 5	MEAN ± S.D.	38.1 ± 27.8	38.5 ± 21.9
BLOCK 6	MEAN ± S.D.	21.6 ± 24.4	31.2 ± 21.8
BLOCK 7	MEAN ± S.D.	11.5 ± 10.9	24.6 ± 20.2
BLOCK 8	MEAN ± S.D.	14.0 ± 12.5	14.6 ± 19.2
BLOCK 9	MEAN ± S.D.	10.5 ± 13.5	15.4 ± 23.8
BLOCK 10	MEAN ± S.D.	2.4 ± 1.9	17.2 ± 23.8
BLOCK 11	MEAN ± S.D.	0.9 ± 0.8	16.2 ± 15.4
BLOCK 12	MEAN ± S.D.	1.2 ± 1.8	15.2 ± 21.2
BLOCK 13	MEAN ± S.D.	3.9 ± 10.2	13.6 ± 19.8
BLOCK 14	MEAN ± S.D.	1.5 ± 2.8	14.8 ± 25.4
BLOCK 15	MEAN ± S.D.	1.0 ± 1.4	11.8 ± 20.7
BLOCK 16	MEAN ± S.D.	1.9 ± 1.6	17.0 ± 16.4
BLOCK 17	MEAN ± S.D.	10.6 ± 23.0	11.6 ± 26.1
BLOCK 18	MEAN ± S.D.	6.6 ± 10.5	7.9 ± 13.5
TOTAL	MEAN ± S.D.	407.0 ± 99.0	495.9 ± 190.1

STATISTICAL ANALYSES OF MOTOR ACTIVITY WERE RESTRICTED TO THE TOTAL NUMBER OF MOVEMENTS AND TOTAL TIME SPENT IN MOVEMENT.

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred once.

b. The test was conducted approximately 5.5 hours postdosage, after completion of the functional observational battery.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Crl:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 2): MOTOR ACTIVITY - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY) <sup>a</sup>		II 0(VEHICLE, CORN OIL)	VI 75(DDT)
TEST SESSION 2b			
NUMBER OF RATS	N	8	8
TIME (SECONDS) SPENT IN MOVEMENT			
BLOCK 1	MEAN ± S.D.	205.0 ± 29.4	171.2 ± 32.1
BLOCK 2	MEAN ± S.D.	185.1 ± 40.7	177.6 ± 30.7
BLOCK 3	MEAN ± S.D.	140.6 ± 54.3	131.4 ± 46.4
BLOCK 4	MEAN ± S.D.	109.6 ± 79.2	87.4 ± 34.1
BLOCK 5	MEAN ± S.D.	77.2 ± 57.7	90.2 ± 66.7
BLOCK 6	MEAN ± S.D.	42.6 ± 52.8	62.8 ± 50.3
BLOCK 7	MEAN ± S.D.	25.2 ± 28.0	50.2 ± 45.6
BLOCK 8	MEAN ± S.D.	29.0 ± 33.1	27.2 ± 42.2
BLOCK 9	MEAN ± S.D.	20.9 ± 34.0	31.8 ± 52.7
BLOCK 10	MEAN ± S.D.	2.1 ± 3.2	37.5 ± 54.5
BLOCK 11	MEAN ± S.D.	0.8 ± 0.9	31.5 ± 32.8
BLOCK 12	MEAN ± S.D.	1.2 ± 2.2	29.9 ± 43.1
BLOCK 13	MEAN ± S.D.	6.8 ± 18.7	33.1 ± 56.6
BLOCK 14	MEAN ± S.D.	2.9 ± 5.8	27.9 ± 49.2
BLOCK 15	MEAN ± S.D.	1.9 ± 2.7	24.6 ± 47.9
BLOCK 16	MEAN ± S.D.	2.2 ± 2.1	36.2 ± 37.2
BLOCK 17	MEAN ± S.D.	19.4 ± 44.6	24.5 ± 58.6
BLOCK 18	MEAN ± S.D.	8.9 ± 14.4	12.9 ± 23.6
TOTAL	MEAN ± S.D.	881.5 ± 235.4	1088.0 ± 446.4

STATISTICAL ANALYSES OF MOTOR ACTIVITY WERE RESTRICTED TO THE TOTAL NUMBER OF MOVEMENTS AND TOTAL TIME SPENT IN MOVEMENT.  
TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred once.

b. The test was conducted approximately 5.5 hours postdosage, after completion of the functional observational battery.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 1): MOTOR ACTIVITY - SUMMARY

DOSAGE GROUP		II	VII
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE, CORN OIL)	200(TRIADIMEFON)
<u>TEST SESSION 2b</u>			
NUMBER OF RATS	N	8	8
NUMBER OF MOVEMENTS			
BLOCK 1	MEAN ± S.D.	86.5 ± 11.7	96.6 ± 6.4
BLOCK 2	MEAN ± S.D.	82.5 ± 13.0	97.8 ± 8.6
BLOCK 3	MEAN ± S.D.	61.1 ± 19.4	94.1 ± 8.3
BLOCK 4	MEAN ± S.D.	51.1 ± 33.0	88.8 ± 8.7
BLOCK 5	MEAN ± S.D.	38.1 ± 27.8	84.9 ± 12.5
BLOCK 6	MEAN ± S.D.	21.6 ± 24.4	86.6 ± 14.8
BLOCK 7	MEAN ± S.D.	11.5 ± 10.9	87.1 ± 10.7
BLOCK 8	MEAN ± S.D.	14.0 ± 12.5	82.4 ± 10.9
BLOCK 9	MEAN ± S.D.	10.5 ± 13.5	81.0 ± 9.9
BLOCK 10	MEAN ± S.D.	2.4 ± 1.9	73.6 ± 10.3
BLOCK 11	MEAN ± S.D.	0.9 ± 0.8	81.8 ± 9.6
BLOCK 12	MEAN ± S.D.	1.2 ± 1.8	82.0 ± 7.8
BLOCK 13	MEAN ± S.D.	3.9 ± 10.2	81.1 ± 14.5
BLOCK 14	MEAN ± S.D.	1.5 ± 2.8	76.9 ± 18.7
BLOCK 15	MEAN ± S.D.	1.0 ± 1.4	73.0 ± 11.4
BLOCK 16	MEAN ± S.D.	1.9 ± 1.6	71.9 ± 13.8
BLOCK 17	MEAN ± S.D.	10.6 ± 23.0	72.6 ± 14.4
BLOCK 18	MEAN ± S.D.	6.6 ± 10.5	72.6 ± 10.7
TOTAL	MEAN ± S.D.	407.0 ± 99.0	1484.8 ± 92.8**

STATISTICAL ANALYSES OF MOTOR ACTIVITY WERE RESTRICTED TO THE TOTAL NUMBER OF MOVEMENTS AND TOTAL TIME SPENT IN MOVEMENT.  
TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred once.

b. The test was conducted approximately 5.5 hours postdosage in Group II and 2 hours postdosage in Group VII, after completion of the functional observational battery.

\*\* Significantly different from the vehicle control group TOTAL ( $P \leq 0.01$ ).

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 2): MOTOR ACTIVITY - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY) <sup>a</sup>		II 0(VEHICLE, CORN OIL)	VII 200(TRIADIMEFON)
<u>TEST SESSION 2b</u>			
NUMBER OF RATS	N	8	8
TIME (SECONDS) SPENT IN MOVEMENT			
BLOCK 1	MEAN ± S.D.	205.0 ± 29.4	248.4 ± 25.7
BLOCK 2	MEAN ± S.D.	185.1 ± 40.7	250.4 ± 14.4
BLOCK 3	MEAN ± S.D.	140.6 ± 54.3	240.6 ± 16.2
BLOCK 4	MEAN ± S.D.	109.6 ± 79.2	239.8 ± 19.0
BLOCK 5	MEAN ± S.D.	77.2 ± 57.7	228.6 ± 37.3
BLOCK 6	MEAN ± S.D.	42.6 ± 52.8	225.5 ± 42.0
BLOCK 7	MEAN ± S.D.	25.2 ± 28.0	227.1 ± 19.1
BLOCK 8	MEAN ± S.D.	29.0 ± 33.1	217.0 ± 40.9
BLOCK 9	MEAN ± S.D.	20.9 ± 34.0	201.2 ± 35.6
BLOCK 10	MEAN ± S.D.	2.1 ± 3.2	196.1 ± 26.8
BLOCK 11	MEAN ± S.D.	0.8 ± 0.9	215.0 ± 21.6
BLOCK 12	MEAN ± S.D.	1.2 ± 2.2	210.0 ± 18.7
BLOCK 13	MEAN ± S.D.	6.8 ± 18.7	225.1 ± 23.2
BLOCK 14	MEAN ± S.D.	2.9 ± 5.8	204.1 ± 65.6
BLOCK 15	MEAN ± S.D.	1.9 ± 2.7	188.0 ± 26.7
BLOCK 16	MEAN ± S.D.	2.2 ± 2.1	188.8 ± 31.5
BLOCK 17	MEAN ± S.D.	19.4 ± 44.6	191.4 ± 38.2
BLOCK 18	MEAN ± S.D.	8.9 ± 14.4	189.6 ± 27.6
TOTAL	MEAN ± S.D.	881.5 ± 235.4	3886.8 ± 282.5**

STATISTICAL ANALYSES OF MOTOR ACTIVITY WERE RESTRICTED TO THE TOTAL NUMBER OF MOVEMENTS AND TOTAL TIME SPENT IN MOVEMENT.  
TOTAL - SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

- a. Dosage occurred once.
  - b. The test was conducted approximately 5.5 hours postdosage in Group II and 2 hours postdosage in Group VII, after completion of the functional observational battery.
- \*\* Significantly different from the vehicle control group TOTAL ( $P \leq 0.01$ ).

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Crl:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 2): MOTOR ACTIVITY - SUMMARY

DOSAGE GROUP	II	VII
DOSAGE (MG/KG/DAY) <sup>a</sup>	0 (VEHICLE, CORN OIL)	200 (TRIADIMEFON)

TEST SESSION 2b

NUMBER OF RATS	N	8	8
TIME (SECONDS) SPENT IN MOVEMENT			
BLOCK 1	MEAN ± S.D.	205.0 ± 29.4	248.4 ± 25.7
BLOCK 2	MEAN ± S.D.	185.1 ± 40.7	250.4 ± 14.4
BLOCK 3	MEAN ± S.D.	140.6 ± 54.3	240.6 ± 16.2
BLOCK 4	MEAN ± S.D.	109.6 ± 79.2	239.8 ± 19.0
BLOCK 5	MEAN ± S.D.	77.2 ± 57.7	228.6 ± 37.3
BLOCK 6	MEAN ± S.D.	42.6 ± 52.8	225.5 ± 42.0
BLOCK 7	MEAN ± S.D.	25.2 ± 28.0	227.1 ± 19.1
BLOCK 8	MEAN ± S.D.	29.0 ± 33.1	217.0 ± 40.9
BLOCK 9	MEAN ± S.D.	20.9 ± 34.0	201.2 ± 35.6
BLOCK 10	MEAN ± S.D.	2.1 ± 3.2	196.1 ± 26.8
BLOCK 11	MEAN ± S.D.	0.8 ± 0.9	215.0 ± 21.6
BLOCK 12	MEAN ± S.D.	1.2 ± 2.2	210.0 ± 18.7
BLOCK 13	MEAN ± S.D.	6.8 ± 18.7	225.1 ± 23.2
BLOCK 14	MEAN ± S.D.	2.9 ± 5.8	204.1 ± 65.6
BLOCK 15	MEAN ± S.D.	1.9 ± 2.7	188.0 ± 26.7
BLOCK 16	MEAN ± S.D.	2.2 ± 2.1	188.8 ± 31.5
BLOCK 17	MEAN ± S.D.	19.4 ± 44.6	191.4 ± 38.2
BLOCK 18	MEAN ± S.D.	8.9 ± 14.4	189.6 ± 27.6
TOTAL	MEAN ± S.D.	881.5 ± 235.4	3886.8 ± 282.5**

STATISTICAL ANALYSES OF MOTOR ACTIVITY WERE RESTRICTED TO THE TOTAL NUMBER OF MOVEMENTS AND TOTAL TIME SPENT IN MOVEMENT.

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred once.

b. The test was conducted approximately 5.5 hours postdosage in Group II and 2 hours postdosage in Group VII, after completion of the functional observational battery.

\*\* Significantly different from the vehicle control group TOTAL ( $P \leq 0.01$ ).



PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 5 (PAGE 1): MOTOR ACTIVITY - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP I 0 (VEHICLE, 0.9% SALINE) MG/KG/DAY a				
RAT #	19761	19762	19782	19787
<u>TEST SESSION 2b</u>				
NUMBER OF MOVEMENTS				
BLOCK 1	76	77	99	95
BLOCK 2	64	84	76	83
BLOCK 3	34	62	54	12
BLOCK 4	10	56	28	0
BLOCK 5	0	35	0	0
BLOCK 6	5	18	0	0
BLOCK 7	0	17	5	0
BLOCK 8	0	0	0	0
BLOCK 9	1	0	0	0
BLOCK 10	0	0	0	0
BLOCK 11	0	0	1	0
BLOCK 12	2	0	0	2
BLOCK 13	2	0	4	0
BLOCK 14	5	0	0	0
BLOCK 15	0	1	1	5
BLOCK 16	1	3	1	0
BLOCK 17	0	2	0	0
BLOCK 18	0	2	3	0
TOTAL	200	357	272	197

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred once daily for nine days.

b. The test was conducted on day 7 of dosage, before dosage and after completion of functional observational battery.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 5 (PAGE 2): MOTOR ACTIVITY - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP I 0 (VEHICLE, 0.9% SALINE) MG/KG/DAY a				
RAT #	19761	19762	19782	19787
<u>TEST SESSION 2b</u>				
TIME (SECONDS) SPENT IN MOVEMENT				
BLOCK 1	166	170	246	233
BLOCK 2	144	176	227	184
BLOCK 3	63	142	112	16
BLOCK 4	16	128	66	0
BLOCK 5	0	82	0	0
BLOCK 6	7	25	0	0
BLOCK 7	0	33	4	0
BLOCK 8	0	0	0	0
BLOCK 9	0	0	0	0
BLOCK 10	0	0	0	0
BLOCK 11	0	0	0	0
BLOCK 12	2	0	0	5
BLOCK 13	0	0	8	0
BLOCK 14	4	0	0	0
BLOCK 15	0	1	1	6
BLOCK 16	1	7	1	0
BLOCK 17	0	0	0	0
BLOCK 18	0	0	2	0
TOTAL	403	764	667	444

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

- Dosage occurred once daily for nine days.
- The test was conducted on day 7 of dosage, before dosage and after completion of functional observational battery.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 5 (PAGE 5): MOTOR ACTIVITY - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP I 0 (VEHICLE, 0.9% SALINE) MG/KG/DAY a				
RAT #	19761	19762	19782	19787
<u>TEST SESSION 3b</u>				
NUMBER OF MOVEMENTS				
BLOCK 1	84	80	81	89
BLOCK 2	94	73	86	22
BLOCK 3	62	80	85	0
BLOCK 4	83	71	61	0
BLOCK 5	67	4	53	0
BLOCK 6	65	0	14	0
BLOCK 7	63	0	7	0
BLOCK 8	28	10	1	0
BLOCK 9	26	5	0	0
BLOCK 10	0	0	7	0
BLOCK 11	0	1	0	22
BLOCK 12	0	0	0	0
BLOCK 13	0	0	0	2
BLOCK 14	0	0	0	0
BLOCK 15	0	0	0	7
BLOCK 16	1	0	0	0
BLOCK 17	1	2	0	0
BLOCK 18	7	0	0	0
TOTAL	581	326	395	142

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

- a. Dosage occurred once daily for nine days.
- b. The test was conducted on day 7 of dosage, before dosage and after completion of functional observational battery.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 5 (PAGE 6): MOTOR ACTIVITY - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP I 0 (VEHICLE, 0.9% SALINE) MG/KG/DAY a				
RAT #	19761	19762	19782	19787
<u>TEST SESSION 3b</u>				
TIME (SECONDS) SPENT IN MOVEMENT				
BLOCK 1	185	181	223	227
BLOCK 2	217	136	240	40
BLOCK 3	151	182	221	0
BLOCK 4	172	160	143	0
BLOCK 5	163	9	118	0
BLOCK 6	137	0	18	0
BLOCK 7	115	0	27	0
BLOCK 8	48	16	0	0
BLOCK 9	47	4	0	0
BLOCK 10	0	0	16	0
BLOCK 11	0	0	0	43
BLOCK 12	0	0	0	0
BLOCK 13	0	0	0	3
BLOCK 14	0	0	0	0
BLOCK 15	0	0	0	16
BLOCK 16	1	0	0	0
BLOCK 17	2	3	0	0
BLOCK 18	8	0	0	0
TOTAL	1246	691	1006	329

TOTAL - SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred once daily for nine days.

b. The test was conducted on day 7 of dosage, before dosage and after completion of functional observational battery.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 6 (PAGE 1): MOTOR ACTIVITY - INDIVIDUAL DATA - FEMALE RATS

DOSAGE GROUP I 0 (VEHICLE, 0.9% SALINE) MG/KG/DAY a				
RAT #	9172	9181	9185	9196
<u>TEST SESSION 2b</u>				
NUMBER OF MOVEMENTS				
BLOCK 1	88	83	95	77
BLOCK 2	67	80	72	82
BLOCK 3	57	45	53	86
BLOCK 4	54	9	39	82
BLOCK 5	63	21	45	37
BLOCK 6	64	0	25	8
BLOCK 7	78	0	4	0
BLOCK 8	71	0	0	0
BLOCK 9	44	0	0	0
BLOCK 10	88	1	0	0
BLOCK 11	61	2	0	0
BLOCK 12	51	1	0	3
BLOCK 13	49	2	0	0
BLOCK 14	52	0	0	0
BLOCK 15	5	0	0	0
BLOCK 16	0	3	3	0
BLOCK 17	0	0	0	0
BLOCK 18	2	0	0	0
TOTAL	894	247	336	375

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

- a. Dosage occurred once daily for nine days.
- b. The test was conducted on day 7 of dosage, before dosage and after completion of functional observational battery.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 6 (PAGE 5): MOTOR ACTIVITY - INDIVIDUAL DATA - FEMALE RATS

DOSAGE GROUP I 0 (VEHICLE, 0.9% SALINE) MG/KG/DAY a				
RAT #	9172	9181	9185	9196
<u>TEST SESSION 3b</u>				
NUMBER OF MOVEMENTS				
BLOCK 1	90	88	80	96
BLOCK 2	84	84	38	68
BLOCK 3	52	37	5	56
BLOCK 4	57	1	0	73
BLOCK 5	35	1	0	52
BLOCK 6	63	1	0	73
BLOCK 7	60	0	0	70
BLOCK 8	81	0	0	27
BLOCK 9	51	7	0	5
BLOCK 10	52	0	0	4
BLOCK 11	32	3	0	0
BLOCK 12	0	0	0	0
BLOCK 13	3	0	0	1
BLOCK 14	0	4	0	5
BLOCK 15	1	0	0	0
BLOCK 16	0	0	0	0
BLOCK 17	0	0	0	4
BLOCK 18	2	4	1	1
TOTAL	663	230	124	535

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred once daily for nine days.

b. The test was conducted on day 7 of dosage, before dosage and after completion of functional observational battery.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 6 (PAGE 6): MOTOR ACTIVITY - INDIVIDUAL DATA - FEMALE RATS

DOSAGE GROUP I 0 (VEHICLE, 0.9% SALINE) MG/KG/DAY a				
RAT #	9172	9181	9185	9196
<u>TEST SESSION 3b</u>				
TIME (SECONDS) SPENT IN MOVEMENT				
BLOCK 1	211	193	196	228
BLOCK 2	196	167	80	163
BLOCK 3	130	73	5	146
BLOCK 4	138	2	0	170
BLOCK 5	79	0	0	138
BLOCK 6	134	1	0	179
BLOCK 7	109	0	0	175
BLOCK 8	178	0	0	60
BLOCK 9	122	8	0	11
BLOCK 10	95	0	0	8
BLOCK 11	49	4	0	0
BLOCK 12	0	0	0	0
BLOCK 13	1	0	0	0
BLOCK 14	0	4	0	4
BLOCK 15	3	0	0	0
BLOCK 16	0	0	0	0
BLOCK 17	0	0	0	3
BLOCK 18	2	5	1	3
TOTAL	1447	457	282	1288

TOTAL - SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred once daily for nine days.

b. The test was conducted on day 7 of dosage, before dosage and after completion of functional observational battery.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - MALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE) 0.9% SALINE a	III 40 ACRYLAMIDE a
RATS EXAMINED b	N	4	4
ALL TISSUES APPEARED NORMAL		4	0**
URINARY BLADDER:			
DISTENDED WITH FLUID, NO BLOCKAGE PRESENT		0	2
DISTENDED WITH URINE, NO BLOCKAGE PRESENT		0	2
BRAIN:			
LEFT CEREBRAL HEMISPHERE; TWO EPIDURAL HEMORRHAGES PRESENT		0	1
CEREBELLUM; ONE EPIDURAL HEMORRHAGE PRESENT		0	1

a. Dosages were administered on days 1 through 9 of the study.

b. Refer to the individual clinical observation table (Table 6) for external observations confirmed at necropsy.

\*\* Significantly different from the vehicle control group value ( $P \leq 0.01$ ).



PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Crl:CD(TM)BR VAF/Plus(TM) RATS

TABLE 7 (PAGE 1): NECROPSY OBSERVATIONS - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RAT NUMBER	DAYS ON STUDY	NUMBER OF DOSAGES	OBSERVATIONS <sup>a</sup>
<b>I</b>				
0 (VEHICLE, 0.9% SALINE) <sup>b</sup>	19761	15	9	ALL TISSUES APPEARED NORMAL.
	19762	15	9	ALL TISSUES APPEARED NORMAL.
	19782	16	9	ALL TISSUES APPEARED NORMAL.
	19787	16	9	ALL TISSUES APPEARED NORMAL.
<b>III</b>				
40 (ACRYLAMIDE) <sup>b</sup>	19764	16	9	URINARY BLADDER: APPROXIMATELY 2.0 ML OF FLUID PRESENT - NO BLOCKAGE PRESENT.
	19768	16	9	URINARY BLADDER: 1.0 ML OF FLUID PRESENT - NO BLOCKAGE PRESENT.
	19770	15	9	URINARY BLADDER: DISTENDED WITH APPROXIMATELY 6 ML OF URINE - NO BLOCKAGE PRESENT. BRAIN: LEFT CEREBRAL HEMISPHERE, TWO EPIDURAL HEMORRHAGES (0.7 X 0.5 CM) PRESENT; CEREBELLUM, ONE EPIDURAL HEMORRHAGE (0.5 CM IN DIAMETER) PRESENT.
	19776	15	9	EXTERNAL OBSERVATION: URINE-STAINED ABDOMINAL FUR. <sup>a</sup> URINARY BLADDER: APPROXIMATELY 3 ML OF URINE PRESENT - NO BLOCKAGE PRESENT.

a. Refer to the individual clinical observation table (Table 6) for external observations confirmed at necropsy.

b. Dosages were administered on days 1 through 9 of the study.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE) 0.9% SALINE a	III 40 ACRYLAMIDE a
RATS EXAMINED b	N	4	4
ALL TISSUES APPEARED NORMAL		4	2
EMACIATION		0	1
URINARY BLADDER:			
DISTENDED WITH FLUID		0	1
DISTENDED WITH URINE		0	1

a. Dosages were administered on days 1 through 9 of the study.

b. Refer to the individual clinical observation table (Table 6) for external observations confirmed at necropsy.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 7 (PAGE 1): NECROPSY OBSERVATIONS - INDIVIDUAL DATA - FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RAT NUMBER	DAYS ON STUDY	NUMBER OF DOSAGES	OBSERVATIONS <sup>a</sup>
<b>I</b>				
0 (VEHICLE, 0.9% SALINE) <sup>b</sup>	9172	16	9	ALL TISSUES APPEARED NORMAL.
	9181	15	9	ALL TISSUES APPEARED NORMAL.
	9185	16	9	ALL TISSUES APPEARED NORMAL.
	9196	15	9	ALL TISSUES APPEARED NORMAL.
<b>III</b>				
40 (ACRYLAMIDE) <sup>b</sup>	9171	16	9	ALL TISSUES APPEARED NORMAL.
	9180	15	9	EMACIATION - LITTLE FAT FOUND AT NECROPSY. URINARY BLADDER: DISTENDED WITH 2.5 ML OF URINE; NO BLOCKAGE PRESENT. ALL OTHER TISSUES APPEARED NORMAL.
	9186	16	9	ALL TISSUES APPEARED NORMAL.
	9191	15	9	URINARY BLADDER: DISTENDED WITH 6 ML OF FLUID; NO BLOCKAGE PRESENT. ALL OTHER TISSUES APPEARED NORMAL.

a. Refer to the individual clinical observation table (Table 6) for external observations confirmed at necropsy.

b. Dosages were administered on days 1 through 9 of the study.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 5 (PAGE 1): MOTOR ACTIVITY - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP I 0 (VEHICLE, 0.9% SALINE) MG/KG/DAY a				
RAT #	19761	19762	19782	19787
<u>TEST SESSION 2b</u>				
NUMBER OF MOVEMENTS				
BLOCK 1	76	77	99	95
BLOCK 2	64	84	76	83
BLOCK 3	34	62	54	12
BLOCK 4	10	56	28	0
BLOCK 5	0	35	0	0
BLOCK 6	5	18	0	0
BLOCK 7	0	17	5	0
BLOCK 8	0	0	0	0
BLOCK 9	1	0	0	0
BLOCK 10	0	0	0	0
BLOCK 11	0	0	1	0
BLOCK 12	2	0	0	2
BLOCK 13	2	0	4	0
BLOCK 14	5	0	0	0
BLOCK 15	0	1	1	5
BLOCK 16	1	3	1	0
BLOCK 17	0	2	0	0
BLOCK 18	0	2	3	0
TOTAL	200	357	272	197

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred once daily for nine days.

b. The test was conducted on day 7 of dosage, before dosage and after completion of functional observational battery.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 5 (PAGE 2): MOTOR ACTIVITY - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP I 0 (VEHICLE, 0.9% SALINE) MG/KG/DAY a				
RAT #	19761	19762	19782	19787
<u>TEST SESSION 2b</u>				
TIME (SECONDS) SPENT IN MOVEMENT				
BLOCK 1	166	170	246	233
BLOCK 2	144	176	227	184
BLOCK 3	63	142	112	16
BLOCK 4	16	128	66	0
BLOCK 5	0	82	0	0
BLOCK 6	7	25	0	0
BLOCK 7	0	33	4	0
BLOCK 8	0	0	0	0
BLOCK 9	0	0	0	0
BLOCK 10	0	0	0	0
BLOCK 11	0	0	0	0
BLOCK 12	2	0	0	5
BLOCK 13	0	0	8	0
BLOCK 14	4	0	0	0
BLOCK 15	0	1	1	6
BLOCK 16	1	7	1	0
BLOCK 17	0	0	0	0
BLOCK 18	0	0	2	0
TOTAL	403	764	667	444

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred once daily for nine days.

b. The test was conducted on day 7 of dosage, before dosage and after completion of functional observational battery.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 5 (PAGE 5): MOTOR ACTIVITY - INDIVIDUAL DATA - MALE RATS

RAT #	DOSAGE GROUP I 0 (VEHICLE, 0.9% SALINE) MG/KG/DAY a			
	19761	19762	19782	19787
<u>TEST SESSION 3b</u>				
NUMBER OF MOVEMENTS				
BLOCK 1	84	80	81	89
BLOCK 2	94	73	86	22
BLOCK 3	62	80	85	0
BLOCK 4	83	71	61	0
BLOCK 5	67	4	53	0
BLOCK 6	65	0	14	0
BLOCK 7	63	0	7	0
BLOCK 8	28	10	1	0
BLOCK 9	26	5	0	0
BLOCK 10	0	0	7	0
BLOCK 11	0	1	0	22
BLOCK 12	0	0	0	0
BLOCK 13	0	0	0	2
BLOCK 14	0	0	0	0
BLOCK 15	0	0	0	7
BLOCK 16	1	0	0	0
BLOCK 17	1	2	0	0
BLOCK 18	7	0	0	0
TOTAL	581	326	395	142

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred daily for three days.

b. The test was conducted on day 4 postdosage after completion of functional observational battery.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 5 (PAGE 6): MOTOR ACTIVITY - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP I 0 (VEHICLE, 0.9% SALINE) MG/KG/DAY a				
RAT #	19761	19762	19782	19787
<u>TEST SESSION 3b</u>				
TIME (SECONDS) SPENT IN MOVEMENT				
BLOCK 1	185	181	223	227
BLOCK 2	217	136	240	40
BLOCK 3	151	182	221	0
BLOCK 4	172	160	143	0
BLOCK 5	163	9	118	0
BLOCK 6	137	0	18	0
BLOCK 7	115	0	27	0
BLOCK 8	48	16	0	0
BLOCK 9	47	4	0	0
BLOCK 10	0	0	16	0
BLOCK 11	0	0	0	43
BLOCK 12	0	0	0	0
BLOCK 13	0	0	0	3
BLOCK 14	0	0	0	0
BLOCK 15	0	0	0	16
BLOCK 16	1	0	0	0
BLOCK 17	2	3	0	0
BLOCK 18	8	0	0	0
TOTAL	1246	691	1006	329

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred daily for three days.

b. The test was conducted on day 4 postdosage after completion of functional observational battery.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 6 (PAGE 1): MOTOR ACTIVITY - INDIVIDUAL DATA - FEMALE RATS

DOSAGE GROUP I 0 (VEHICLE, 0.9% SALINE) MG/KG/DAY a				
RAT #	9172	9181	9185	9196
<u>TEST SESSION 2b</u>				
NUMBER OF MOVEMENTS				
BLOCK 1	88	83	95	77
BLOCK 2	67	80	72	82
BLOCK 3	57	45	53	86
BLOCK 4	54	9	39	82
BLOCK 5	63	21	45	37
BLOCK 6	64	0	25	8
BLOCK 7	78	0	4	0
BLOCK 8	71	0	0	0
BLOCK 9	44	0	0	0
BLOCK 10	88	1	0	0
BLOCK 11	61	2	0	0
BLOCK 12	51	1	0	3
BLOCK 13	49	2	0	0
BLOCK 14	52	0	0	0
BLOCK 15	5	0	0	0
BLOCK 16	0	3	3	0
BLOCK 17	0	0	0	0
BLOCK 18	2	0	0	0
TOTAL	894	247	336	375

TOTAL - SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred once daily for nine days.

b. The test was conducted on day 7 of dosage, before dosage and after completion of functional observational battery.



PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 6 (PAGE 2): MOTOR ACTIVITY - INDIVIDUAL DATA - FEMALE RATS

DOSAGE GROUP I 0 (VEHICLE, 0.9% SALINE) MG/KG/DAY a				
RAT #	9172	9181	9185	9196
<u>TEST SESSION 2b</u>				
TIME (SECONDS) SPENT IN MOVEMENT				
BLOCK 1	218	211	202	206
BLOCK 2	130	166	189	164
BLOCK 3	132	68	111	205
BLOCK 4	104	12	79	190
BLOCK 5	129	34	85	66
BLOCK 6	138	0	41	15
BLOCK 7	177	0	2	0
BLOCK 8	158	0	0	0
BLOCK 9	86	0	0	0
BLOCK 10	165	2	0	0
BLOCK 11	140	5	0	0
BLOCK 12	121	1	0	5
BLOCK 13	94	1	0	0
BLOCK 14	90	0	0	0
BLOCK 15	8	0	0	0
BLOCK 16	0	2	9	0
BLOCK 17	0	0	0	0
BLOCK 18	1	0	0	0
TOTAL	1891	502	718	851

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred once daily for nine days.

b. The test was conducted on day 7 of dosage, before dosage and after completion of functional observational battery.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 6 (PAGE 5): MOTOR ACTIVITY - INDIVIDUAL DATA - FEMALE RATS

DOSAGE GROUP I 0 (VEHICLE, 0.9% SALINE) MG/KG/DAY a				
RAT #	9172	9181	9185	9196
<b>TEST SESSION 3b</b>				
<b>NUMBER OF MOVEMENTS</b>				
BLOCK 1	90	88	80	96
BLOCK 2	84	84	38	68
BLOCK 3	52	37	5	56
BLOCK 4	57	1	0	73
BLOCK 5	35	1	0	52
BLOCK 6	63	1	0	73
BLOCK 7	60	0	0	70
BLOCK 8	81	0	0	27
BLOCK 9	51	7	0	5
BLOCK 10	52	0	0	4
BLOCK 11	32	3	0	0
BLOCK 12	0	0	0	0
BLOCK 13	3	0	0	1
BLOCK 14	0	4	0	5
BLOCK 15	1	0	0	0
BLOCK 16	0	0	0	0
BLOCK 17	0	0	0	4
BLOCK 18	2	4	1	1
TOTAL	663	230	124	535

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred daily for three days.

b. The test was conducted on day 4 postdosage after completion of functional observational battery.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 6 (PAGE 6): MOTOR ACTIVITY - INDIVIDUAL DATA - FEMALE RATS

DOSAGE GROUP I 0 (VEHICLE, 0.9% SALINE) MG/KG/DAY a				
RAT #	9172	9181	9185	9196
<u>TEST SESSION 3b</u>				
TIME (SECONDS) SPENT IN MOVEMENT				
BLOCK 1	211	193	196	228
BLOCK 2	196	167	80	163
BLOCK 3	130	73	5	145
BLOCK 4	138	2	0	170
BLOCK 5	79	0	0	138
BLOCK 6	134	1	0	179
BLOCK 7	109	0	0	175
BLOCK 8	178	0	0	60
BLOCK 9	122	8	0	11
BLOCK 10	95	0	0	8
BLOCK 11	49	4	0	0
BLOCK 12	0	0	0	0
BLOCK 13	1	0	0	0
BLOCK 14	0	4	0	4
BLOCK 15	3	0	0	0
BLOCK 16	0	0	0	0
BLOCK 17	0	0	0	3
BLOCK 18	2	5	1	3
TOTAL	1447	457	282	1288

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred daily for three days.

b. The test was conducted on day 4 postdosage after completion of functional observational battery.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - MALE RATS

DOSAGE GROUP		I	IV
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	200
		0.9% SALINE a	IDPN b
RATS EXAMINED	N	4	4
ALL TISSUES APPEARED NORMAL		4	4

a. Dosages were administered on days 1 through 9 of the study.

b. Dosages were administered on days 1 through 3 of the study.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 7 (PAGE 1): NECROPSY OBSERVATIONS - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RAT NUMBER	DAYS ON STUDY	NUMBER OF DOSAGES	OBSERVATIONS
<b>I</b>				
0 (VEHICLE, 0.9% SALINE) a	19761	15	9	ALL TISSUES APPEARED NORMAL.
	19762	15	9	ALL TISSUES APPEARED NORMAL.
	19782	16	9	ALL TISSUES APPEARED NORMAL.
	19787	16	9	ALL TISSUES APPEARED NORMAL.
<b>IV</b>				
200 (IDPN) b	19775	15	3	ALL TISSUES APPEARED NORMAL.
	19780	15	3	ALL TISSUES APPEARED NORMAL.
	19784	16	3	ALL TISSUES APPEARED NORMAL.
	19788	16	3	ALL TISSUES APPEARED NORMAL.

a. Dosages were administered on days 1 through 9 of the study.

b. Dosages were administered on days 1 through 3 of the study.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - FEMALE RATS

DOSAGE GROUP		I	IV
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	200
		0.9% SALINE a	IDPN b
RATS EXAMINED	N	4	4
ALL TISSUES APPEARED NORMAL		4	4

a. Dosages were administered on days 1 through 9 of the study.

b. Dosages were administered on days 1 through 3 of the study.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 7 (PAGE 1): NECROPSY OBSERVATIONS - INDIVIDUAL DATA - FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RAT NUMBER	DAYS ON STUDY	NUMBER OF DOSAGES	OBSERVATIONS
<b>I</b>				
0 (VEHICLE, 0.9% SALINE) a	9172	16	9	ALL TISSUES APPEARED NORMAL.
	9181	15	9	ALL TISSUES APPEARED NORMAL.
	9185	16	9	ALL TISSUES APPEARED NORMAL.
	9196	15	9	ALL TISSUES APPEARED NORMAL.
<b>IV</b>				
200 (IDPN) b	9174	15	3	ALL TISSUES APPEARED NORMAL.
	9177	15	3	ALL TISSUES APPEARED NORMAL.
	9183	15	3	ALL TISSUES APPEARED NORMAL.
	9187	15	3	ALL TISSUES APPEARED NORMAL.

a. Dosages were administered on days 1 through 9 of the study.

b. Dosages were administered on days 1 through 3 of the study.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 3 (PAGE 1): FUNCTIONAL OBSERVATIONAL BATTERY - INDIVIDUAL DATA - MALE RATS

TEST SESSION 2a

RAT #	19765	19767	19777	19785	19769	19771	19773	19781
DOSAGE GROUP b	II	II	II	II	V	V	V	V
HOME CAGE BEHAVIOR	2	2	1	1	2	2	3	1
BEHAVIORAL OR POSTURAL ALTERATIONS c	1	1	1	1	4	1	4	1
REACTION TO REMOVAL	1	1	1	1	1	1	1	1
REACTION TO HANDLING	1	1	1	1	1	1	1	1
REARS	0	1	1	0	0	0	0	1
FECAL BOLUSES	4	0	0	0	0	0	0	0
URINE POOLS	0	2	1	1	1	4	2	3
LEVEL OF AROUSAL	3	3	3	2	2	2	2	3
BEHAVIORAL OR POSTURAL ALTERATIONS d	1	1	1	1	4	4	4	1
GAIT PATTERN	1	1	1	1	1	3	1	1
SEVERITY OF GAIT ABNORMALITY	1	1	1	1	1	3	1	1
PALPEBRAL CLOSURE	1	2	2	3	1	1	1	1
PROMINENCE OF THE EYE	1	1	1	1	1	1	1	1
LACRIMATION	1	1	1	1	1	3	2	1
SALIVATION	1	1	1	1	2	4	4	3
PILOERECTION	0	0	0	0	0	0	0	0
RESPIRATION	0	0	0	1	0	1	1	0
APPEARANCE	1	1	1	1	1	1	1	1
REACTION TO VISUAL STIMULUS	1	2	2	1	2	1	1	1
REACTION TO TACTILE STIMULUS	2	1	1	1	1	1	2	2
REACTION TO AUDITORY STIMULUS	3	3	3	3	1	1	3	3
REACTION TO TAIL PINCH	4	2	3	2	1	1	2	1
VISUAL PLACING RESPONSE	1	1	1	1	2	1	2	1
AIR RIGHTING RESPONSE	1	1	1	2	3	2	2	1
PUPILLARY RESPONSE	1	1	1	0	0e	0e	0e	1
FORELIMB GRIP STRENGTH (G) #1	575	700	825	775	750	325	1075	600
FORELIMB GRIP STRENGTH (G) #2	500	600	650	650	325	400	650	625
HINDLIMB GRIP STRENGTH (G) #1	850	825	400	850	975	550	900	450
HINDLIMB GRIP STRENGTH (G) #2	925	650	600	775	775	550	1150	350
LANDING FOOT SPREAD (CM) #1	8.6	5.5	6.8	6.5	10.0	6.8	12.0	8.3
LANDING FOOT SPREAD (CM) #2	8.0	7.5	7.0	8.2	8.7	8.8	11.0	7.8
BODY WEIGHT	479	491	530	443	475	511	549	446

#1 = FIRST MEASUREMENT

#2 = SECOND MEASUREMENT

a. The examination was conducted approximately 5.5 hours postdosage in Group II and approximately 1 hour postdosage in Group V.

b. Dosage occurred once.

c. Observation made in the home cage.

d. Observation made in the open field.

e. Miosis identified.



PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 4 (PAGE 1): FUNCTIONAL OBSERVATIONAL BATTERY - INDIVIDUAL DATA - FEMALE RATS

TEST SESSION 2a

RAT #	9176	9189	9190	9197	9173	9175	9184	9198
DOSAGE GROUP b	II	II	II	II	V	V	V	V
HOME CAGE BEHAVIOR	2	1	2	2	2	2	1	2
BEHAVIORAL OR POSTURAL ALTERATIONS c	1	1	1	1	1	1	1	5
REACTION TO REMOVAL	1	1	3	1	1	1	1	1
REACTION TO HANDLING	1	1	1	1	1	1	1	1
REARS	1	0	0	0	0	2	0	0
FECAL BOLUSES	0	0	5	0	0	0	0	0
URINE POOLS	0	1	2	1	6	3	2	2
LEVEL OF AROUSAL	3	3	2	2	2	3	2	2
BEHAVIORAL OR POSTURAL ALTERATIONS d	1	5	1	1	4	4	4	4
GAIT PATTERN	1	1	1	1	1	4	1	1
SEVERITY OF GAIT ABNORMALITY	1	1	1	1	1	3	1	1
PALPEBRAL CLOSURE	1	1	2	2	1	1	1	1
PROMINENCE OF THE EYE	1	1	1	1	1	1	1	1
LACRIMATION	1	1	1	1	2	2	1	3
SALIVATION	1	1	1	1	4	1	1	3
PILOBRECTION	0	0	0	0	0	0	0	0
RESPIRATION	0	0	0	1	1	0	0	0
APPEARANCE	1	1	1	1	3	1	1	1
REACTION TO VISUAL STIMULUS	1	2	1	3	1	1	2	1
REACTION TO TACTILE STIMULUS	2	1	2	2	1	1	1	1
REACTION TO AUDITORY STIMULUS	3	3	3	3	1	2	3	3
REACTION TO TAIL PINCH	4	2	1	2	1	1	1	1
VISUAL PLACING RESPONSE	1	1	1	1	2	2	1	2
AIR RIGHTING RESPONSE	1	1	1	1	1	1	2	3
PUPILLARY RESPONSE	1	1	1	1	0e	1	1	1e
FORELIMB GRIP STRENGTH (G) #1	550	450	525	1300	425	300	625	300
FORELIMB GRIP STRENGTH (G) #2	350	425	475	950	575	750	550	200
HINDLIMB GRIP STRENGTH (G) #1	550	500	775	600	700	550	375	525
HINDLIMB GRIP STRENGTH (G) #2	450	450	575	525	625	925	525	775
LANDING FOOT SPREAD (CM) #1	4.6	7.9	7.4	7.6	9.3	5.1	4.9	6.8
LANDING FOOT SPREAD (CM) #2	7.0	6.0	5.8	8.0	8.6	6.0	7.0	7.9
BODY WEIGHT	274	380	279	308	237	272	313	341

#1 - FIRST MEASUREMENT

#2 - SECOND MEASUREMENT

a. The examination was conducted approximately 5.5 hours postdosage in Group II and approximately 1 hour postdosage in Group V.

b. Dosage occurred once.

c. Observation made in the home cage.

d. Observation made in the open field.

e. Miosis identified.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Crl:CD(TM)BR VAF/Plus(TM) RATS

TABLE 5 (PAGE 1): MOTOR ACTIVITY - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP II 0 (VEHICLE, CORN OIL) MG/KG/DAY a				
RAT #	19765	19767	19777	19785
<u>TEST SESSION 2b</u>				
NUMBER OF MOVEMENTS				
BLOCK 1	100	91	82	94
BLOCK 2	85	94	73	85
BLOCK 3	86	84	35	75
BLOCK 4	71	71	0	78
BLOCK 5	62	74	0	56
BLOCK 6	53	65	0	17
BLOCK 7	10	18	0	13
BLOCK 8	24	5	1	6
BLOCK 9	23	0	2	2
BLOCK 10	0	5	4	1
BLOCK 11	0	0	1	1
BLOCK 12	0	1	0	1
BLOCK 13	0	0	0	0
BLOCK 14	0	0	0	0
BLOCK 15	0	2	4	1
BLOCK 16	3	3	0	0
BLOCK 17	2	1	9	0
BLOCK 18	0	2	24	0
TOTAL	519	516	235	430

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred once.

b. The test was conducted approximately 5.5 hours postdosage, after functional observational battery on the day of dosage.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 5 (PAGE 2): MOTOR ACTIVITY - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP II 0 (VEHICLE, CORN OIL) MG/KG/DAY <sup>a</sup>				
RAT #	19765	19767	19777	19785
<u>TEST SESSION 2b</u>				
TIME (SECONDS) SPENT IN MOVEMENT				
BLOCK 1	207	212	183	209
BLOCK 2	238	213	130	170
BLOCK 3	206	213	81	166
BLOCK 4	165	163	0	170
BLOCK 5	114	154	0	127
BLOCK 6	137	112	0	40
BLOCK 7	16	41	0	19
BLOCK 8	49	7	1	6
BLOCK 9	46	0	1	1
BLOCK 10	0	9	4	1
BLOCK 11	0	0	0	1
BLOCK 12	0	1	0	0
BLOCK 13	0	0	0	0
BLOCK 14	0	0	0	0
BLOCK 15	0	2	3	8
BLOCK 16	3	3	0	0
BLOCK 17	1	2	16	0
BLOCK 18	0	2	31	0
TOTAL	1182	1134	450	918

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred once.

b. The test was conducted approximately 5.5 hours postdosage, after functional observational battery on the day of dosage.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 6 (PAGE 1): MOTOR ACTIVITY - INDIVIDUAL DATA - FEMALE RATS

DOSAGE GROUP II 0 (VEHICLE, CORN OIL) MG/KG/DAY a				
RAT #	9176	9189	9190	9197
<u>TEST SESSION 2b</u>				
NUMBER OF MOVEMENTS				
BLOCK 1	93	62	81	89
BLOCK 2	99	71	61	92
BLOCK 3	49	65	54	41
BLOCK 4	6	44	50	89
BLOCK 5	1	24	36	52
BLOCK 6	1	5	12	20
BLOCK 7	0	0	25	26
BLOCK 8	23	0	33	20
BLOCK 9	38	0	11	8
BLOCK 10	4	2	0	3
BLOCK 11	2	1	0	2
BLOCK 12	5	0	0	3
BLOCK 13	0	29	0	2
BLOCK 14	0	7	0	5
BLOCK 15	0	0	1	0
BLOCK 16	4	1	1	3
BLOCK 17	67	0	2	4
BLOCK 18	23	0	0	4
TOTAL	415	311	367	463

TOTAL - SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred once.

b. The test was conducted approximately 5.5 hours postdosage, after functional observational battery on the day of dosage.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Crl:CD(TM)BR VAF/Plus(TM) RATS

TABLE 6 (PAGE 2): MOTOR ACTIVITY - INDIVIDUAL DATA - FEMALE RATS

DOSAGE GROUP II 0 (VEHICLE, CORN OIL) MG/KG/DAY a				
RAT #	9176	9189	9190	9197
<u>TEST SESSION 2b</u>				
TIME (SECONDS) SPENT IN MOVEMENT				
BLOCK 1	259	166	179	225
BLOCK 2	225	167	135	203
BLOCK 3	96	164	122	77
BLOCK 4	7	85	77	210
BLOCK 5	0	57	59	107
BLOCK 6	1	7	15	29
BLOCK 7	0	0	76	50
BLOCK 8	49	0	94	26
BLOCK 9	96	0	11	12
BLOCK 10	0	0	0	3
BLOCK 11	2	1	0	2
BLOCK 12	6	0	0	3
BLOCK 13	0	53	0	1
BLOCK 14	0	16	0	7
BLOCK 15	0	0	2	0
BLOCK 16	6	1	1	4
BLOCK 17	129	0	4	3
BLOCK 18	33	0	0	5
TOTAL	909	717	775	967

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred once.

b. The test was conducted approximately 5.5 hours postdosage, after functional observational battery on the day of dosage.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - MALE RATS

DOSAGE GROUP		II	V
DOSAGE (MG/KG/DAY)		0 (VEHICLE) CORN OIL <sup>a</sup>	75 CARBARYL <sup>a</sup>
RATS EXAMINED	N	4	4
ALL TISSUES APPEARED NORMAL		4	4

<sup>a</sup>. Dosage was administered on day 1 of the study.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 7 (PAGE 1): NECROPSY OBSERVATIONS - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RAT NUMBER	DAYS ON STUDY	NUMBER OF DOSAGES	OBSERVATIONS
II				
0 (VEHICLE, CORN OIL) a	19765	15	1	ALL TISSUES APPEARED NORMAL.
	19767	13	1	ALL TISSUES APPEARED NORMAL.
	19777	13	1	ALL TISSUES APPEARED NORMAL.
	19785	15	1	ALL TISSUES APPEARED NORMAL.
V				
75 (CARBARYL) a	19769	8	1	ALL TISSUES APPEARED NORMAL.
	19771	7	1	ALL TISSUES APPEARED NORMAL.
	19773	8	1	ALL TISSUES APPEARED NORMAL.
	19781	7	1	ALL TISSUES APPEARED NORMAL.

a. Dosage was administered on day 1 of the study.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)		II 0(VEHICLE) CORN OIL a	V 75 CARBARYL a
RATS EXAMINED	N	4	4
ALL TISSUES APPEARED NORMAL		4	4

a. Dosage was administered on day 1 of the study.



PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 7 (PAGE 1): NECROPSY OBSERVATIONS - INDIVIDUAL DATA - FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RAT NUMBER	DAYS ON STUDY	NUMBER OF DOSAGES	OBSERVATIONS
II				
0 (VEHICLE, CORN OIL) a	9176	13	1	ALL TISSUES APPEARED NORMAL.
	9189	15	1	ALL TISSUES APPEARED NORMAL.
	9190	15	1	ALL TISSUES APPEARED NORMAL.
	9197	13	1	ALL TISSUES APPEARED NORMAL.
V				
75 (CARBARYL) a	9173	7	1	ALL TISSUES APPEARED NORMAL.
	9175	7	1	ALL TISSUES APPEARED NORMAL.
	9184	8	1	ALL TISSUES APPEARED NORMAL.
	9198	7	1	ALL TISSUES APPEARED NORMAL.

a. Dosage was administered on day 1 of the study.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN CrI:CD(TM)BR VAF/Plus(TM) RATS

TABLE 3 (PAGE 1): FUNCTIONAL OBSERVATIONAL BATTERY - INDIVIDUAL DATA - MALE RATS

TEST SESSION 2a

RAT #	19765	19767	19777	19785	19778	19779	19783	19786
DOSAGE GROUP b	II	II	II	II	VI	VI	VI	VI
HOME CAGE BEHAVIOR	2	2	1	1	3	2	1	2
BEHAVIORAL OR POSTURAL ALTERATIONS c	1	1	1	1	1	1	1	5
REACTION TO REMOVAL	2	1	1	1	1	1	3	1
REACTION TO HANDLING	1	1	1	1	1	1	1	1
REARS	0	1	1	0	1	4	2	3
FECAL BOLUSES	4	0	0	0	1	4	1	0
URINE POOLS	0	2	1	1	1	3	3	3
LEVEL OF AROUSAL	3	3	3	2	3	3	3	3
BEHAVIORAL OR POSTURAL ALTERATIONS d	1	1	1	1	1	1	4	5
GAIT PATTERN	1	1	1	1	1	1	1	1
SEVERITY OF GAIT ABNORMALITY	1	1	1	1	1	1	1	1
PALPEBRAL CLOSURE	1	2	2	3	1	1	2	1
PROMINENCE OF THE EYE	1	1	1	1	1	1	1	1
LACRIMATION	1	1	1	1	1	1	1	1
SALIVATION	1	1	1	1	1	1	1	1
PILOERECTION	0	0	0	0	0	0	0	0
RESPIRATION	0	0	0	1	0	0	0	0
APPEARANCE	1	1	1	1	1	1	1	1
REACTION TO VISUAL STIMULUS	1	2	2	1	2	2	1	1
REACTION TO TACTILE STIMULUS	2	1	1	1	1	2	2	1
REACTION TO AUDITORY STIMULUS	3	3	3	3	3	3	3	3
REACTION TO TAIL PINCH	4	2	3	2	2	4	2	2
VISUAL PLACING RESPONSE	1	1	1	1	1	1	1	1
AIR RIGHTING RESPONSE	1	1	1	2	1	2	1	1
PUPILLARY RESPONSE	1	1	1	1	1	1	1e	1
FORELIMB GRIP STRENGTH (G) #1	575	700	825	775	600	675	1025	425
FORELIMB GRIP STRENGTH (G) #2	500	600	650	650	750	575	775	325
HINDLIMB GRIP STRENGTH (G) #1	850	825	400	850	625	650	575	500
HINDLIMB GRIP STRENGTH (G) #2	925	650	600	775	750	800	575	250
LANDING FOOT SPLAY (CM) #1	8.6	5.5	6.8	6.5	10.0	10.0	8.2	5.1
LANDING FOOT SPLAY (CM) #2	8.0	7.5	7.0	8.2	8.0	7.5	9.0	8.0
BODY WEIGHT	479	491	530	443	464	493	498	353

#1 - FIRST MEASUREMENT

#2 - SECOND MEASUREMENT

- The examination was conducted approximately 5.5 hours postdosage.
- Dosage occurred once.
- Observation made in the home cage.
- Observation made in the open field.
- Miosis identified.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 4 (PAGE 1): FUNCTIONAL OBSERVATIONAL BATTERY - INDIVIDUAL DATA - FEMALE RATS

TEST SESSION 2a

RAT #	9176	9189	9190	9197	9178	9188	9193	9194
DOSAGE GROUP b	II	II	II	II	VI	VI	VI	VI
HOME CAGE BEHAVIOR	2	1	2	2	1	2	1	2
BEHAVIORAL OR POSTURAL ALTERATIONS c	1	1	1	1	1	1	1	5
REACTION TO REMOVAL	1	1	3	1	1	1	1	1
REACTION TO HANDLING	1	1	1	1	1	1	1	1
REARS	1	0	0	0	1	0	0	0
FECAL BOLUSES	0	0	5	0	1	0	0	0
URINE POOLS	0	1	2	1	2	3	2	0
LEVEL OF AROUSAL	3	3	2	2	3	3	3	3
BEHAVIORAL OR POSTURAL ALTERATIONS d	1	1	1	1	1	5	1	5
GAIT PATTERN	1	1	1	1	1	1	1	1
SEVERITY OF GAIT ABNORMALITY	1	1	1	1	1	1	1	1
PALPEBRAL CLOSURE	1	1	2	2	1	1	1	1
PROMINENCE OF THE EYE	1	1	1	1	1	1	1	1
LACRIMATION	1	1	1	1	1	1	1	1
SALIVATION	1	1	1	1	1	1	1	1
PILOERECTOR	0	0	0	0	0	0	0	0
RESPIRATION	0	0	0	1	0	0	0	0
APPEARANCE	1	1	1	1	1	1	1	1
REACTION TO VISUAL STIMULUS	1	2	1	3	1	1	2	2
REACTION TO TACTILE STIMULUS	2	1	2	2	2	1	2	1
REACTION TO AUDITORY STIMULUS	3	3	3	3	2	3	3	3
REACTION TO TAIL PINCH	4	2	1	2	3	2	2	4
VISUAL PLACING RESPONSE	1	1	1	1	1	1	1	1
AIR RIGHTING RESPONSE	1	1	1	1	1	1	1	1
PUPILLARY RESPONSE	1	1	1	1	1	1	1	1
FORELIMB GRIP STRENGTH (G) #1	550	450	525	1300	625	625	325	575
FORELIMB GRIP STRENGTH (G) #2	350	425	475	950	500	350	300	950
HINDLIMB GRIP STRENGTH (G) #1	550	500	775	600	525	775	450	375
HINDLIMB GRIP STRENGTH (G) #2	450	450	575	525	425	550	700	375
LANDING FOOT SPREAD (CM) #1	4.6	7.9	7.4	7.6	7.5	4.1	6.8	6.5
LANDING FOOT SPREAD (CM) #2	7.0	6.0	5.8	8.0	7.5	5.3	8.4	7.8
BODY WEIGHT	274	380	279	308	258	309	288	325

#1 - FIRST MEASUREMENT

#2 - SECOND MEASUREMENT

- a. The examination was conducted approximately 5.5 hours postdosage.
- b. Dosage occurred once.
- c. Observation made in the home cage.
- d. Observation made in the open field.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 5 (PAGE 1): MOTOR ACTIVITY - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP II 0 (VEHICLE, CORN OIL) MG/KG/DAY a				
RAT #	19765	19767	19777	19785
<b>TEST SESSION 2b</b>				
NUMBER OF MOVEMENTS				
BLOCK 1	100	91	82	94
BLOCK 2	85	94	73	85
BLOCK 3	86	84	35	75
BLOCK 4	71	71	0	78
BLOCK 5	62	74	0	56
BLOCK 6	53	65	0	17
BLOCK 7	10	18	0	13
BLOCK 8	24	5	1	6
BLOCK 9	23	0	2	2
BLOCK 10	0	5	4	1
BLOCK 11	0	0	1	1
BLOCK 12	0	1	0	1
BLOCK 13	0	0	0	0
BLOCK 14	0	0	0	0
BLOCK 15	0	2	4	1
BLOCK 16	3	3	0	0
BLOCK 17	2	1	9	0
BLOCK 18	0	2	24	0
TOTAL	519	516	235	430

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred once.

b. The test was conducted approximately 5.5 hours postdosage, after functional observational battery on the day of dosage.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 5 (PAGE 2): MOTOR ACTIVITY - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP II 0 (VEHICLE, CORN OIL) MG/KG/DAY a				
RAT #	19765	19767	19777	19785
<u>TEST SESSION 2b</u>				
TIME (SECONDS) SPENT IN MOVEMENT				
BLOCK 1	207	212	183	209
BLOCK 2	238	213	130	170
BLOCK 3	206	213	81	166
BLOCK 4	165	163	0	170
BLOCK 5	114	154	0	127
BLOCK 6	137	112	0	40
BLOCK 7	16	41	0	19
BLOCK 8	49	7	1	6
BLOCK 9	46	0	1	1
BLOCK 10	0	9	4	1
BLOCK 11	0	0	0	1
BLOCK 12	0	1	0	0
BLOCK 13	0	0	0	0
BLOCK 14	0	0	0	0
BLOCK 15	0	2	3	8
BLOCK 16	3	3	0	0
BLOCK 17	1	2	16	0
BLOCK 18	0	2	31	0
TOTAL	1182	1134	450	918

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred once.

b. The test was conducted approximately 5.5 hours postdosage, after functional observational battery on the day of dosage.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 6 (PAGE 1): MOTOR ACTIVITY - INDIVIDUAL DATA - FEMALE RATS

DOSAGE GROUP II 0 (VEHICLE, CORN OIL) MG/KG/DAY a				
RAT #	9176	9189	9190	9197
<u>TEST SESSION 2b</u>				
NUMBER OF MOVEMENTS				
BLOCK 1	93	62	81	89
BLOCK 2	99	71	61	92
BLOCK 3	49	65	54	41
BLOCK 4	6	44	50	89
BLOCK 5	1	24	36	52
BLOCK 6	1	5	12	20
BLOCK 7	0	0	25	26
BLOCK 8	23	0	33	20
BLOCK 9	38	0	11	8
BLOCK 10	4	2	0	3
BLOCK 11	2	1	0	2
BLOCK 12	5	0	0	3
BLOCK 13	0	29	0	2
BLOCK 14	0	7	0	5
BLOCK 15	0	0	1	0
BLOCK 16	4	1	1	3
BLOCK 17	67	0	2	4
BLOCK 18	23	0	0	4
TOTAL	415	311	367	463

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred once.

b. The test was conducted approximately 5.5 hours postdosage, after functional observational battery on the day of dosage.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 6 (PAGE 2): MOTOR ACTIVITY - INDIVIDUAL DATA - FEMALE RATS

DOSAGE GROUP II 0 (VEHICLE, CORN OIL) MG/KG/DAY <sup>a</sup>				
RAT #	9176	9189	9190	9197
<u>TEST SESSION 2b</u>				
TIME (SECONDS) SPENT IN MOVEMENT				
BLOCK 1	259	166	179	225
BLOCK 2	225	167	135	203
BLOCK 3	96	164	122	77
BLOCK 4	7	85	77	210
BLOCK 5	0	57	59	107
BLOCK 6	1	7	15	29
BLOCK 7	0	0	76	50
BLOCK 8	49	0	94	26
BLOCK 9	96	0	11	12
BLOCK 10	0	0	0	3
BLOCK 11	2	1	0	2
BLOCK 12	6	0	0	3
BLOCK 13	0	53	0	1
BLOCK 14	0	16	0	7
BLOCK 15	0	0	2	0
BLOCK 16	6	1	1	4
BLOCK 17	129	0	4	3
BLOCK 18	33	0	0	5
TOTAL	909	717	775	967

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred once.

b. The test was conducted approximately 5.5 hours postdosage, after functional observational battery on the day of dosage.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - MALE RATS

DOSAGE GROUP		II	VI
DOSAGE (MG/KG/DAY)		0(VEHICLE)	75
		CORN OIL a	DDT a
RATS EXAMINED	N	4	4
ALL TISSUES APPEARED NORMAL		4	4

a. Dosage was administered on day 1 of the study.



PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 7 (PAGE 1): NECROPSY OBSERVATIONS - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RAT NUMBER	DAYS ON STUDY	NUMBER OF DOSAGES	OBSERVATIONS <sup>a</sup>
II				
0 (VEHICLE, CORN OIL) <sup>b</sup>	19765	15	1	ALL TISSUES APPEARED NORMAL.
	19767	13	1	ALL TISSUES APPEARED NORMAL.
	19777	13	1	ALL TISSUES APPEARED NORMAL.
	19785	15	1	ALL TISSUES APPEARED NORMAL.
VI				
75 (DDT) <sup>b</sup>	19778	13	1	EXTERNAL OBSERVATIONS: EYES: BOTH, CHROMODACRYORRHEA. <sup>a</sup> INCISORS: UPPER LEFT, MISALIGNED <sup>a</sup> ; UPPER RIGHT, MISSING. <sup>a</sup>
	19779	13	1	ALL TISSUES APPEARED NORMAL.
	19783	15	1	ALL TISSUES APPEARED NORMAL.
	19786	15	1	ALL TISSUES APPEARED NORMAL.

<sup>a</sup>. Refer to the individual clinical observation table (Table 6) for external observations confirmed at necropsy.

<sup>b</sup>. Dosage was administered on day 1 of the study.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - FEMALE RATS

DOSAGE GROUP		II	VI
DOSAGE (MG/KG/DAY)		0 (VEHICLE) CORN OIL a	75 DDT a
RATS EXAMINED	N	4	4
ALL TISSUES APPEARED NORMAL		4	4

a. Dosage was administered on day 1 of the study.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 7 (PAGE 1): NECROPSY OBSERVATIONS - INDIVIDUAL DATA - FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RAT NUMBER	DAYS ON STUDY	NUMBER OF DOSAGES	OBSERVATIONS
II				
0 (VEHICLE, CORN OIL) a	9176	13	1	ALL TISSUES APPEARED NORMAL.
	9189	15	1	ALL TISSUES APPEARED NORMAL.
	9190	15	1	ALL TISSUES APPEARED NORMAL.
	9197	13	1	ALL TISSUES APPEARED NORMAL.
VI				
75 (DDT) a	9178	13	1	ALL TISSUES APPEARED NORMAL.
	9188	13	1	ALL TISSUES APPEARED NORMAL.
	9193	15	1	ALL TISSUES APPEARED NORMAL.
	9194	15	1	ALL TISSUES APPEARED NORMAL.

a. Dosage was administered on day 1 of the study.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Crl:CD(TM)BR VAF/Plus(TM) RATS

TABLE 3 (PAGE 1): FUNCTIONAL OBSERVATIONAL BATTERY - INDIVIDUAL DATA - MALE RATS

TEST SESSION 2a

RAT #	19765	19767	19777	19785	19763	19766	19772	19774
DOSAGE GROUP b	II	II	II	II	VII	VII	VII	VII
HOME CAGE BEHAVIOR	2	2	1	1	3	3	2	3
BEHAVIORAL OR POSTURAL ALTERATIONS c	1	1	1	1	1	1	1	1
REACTION TO REMOVAL	1	1	1	1	1	1	1	1
REACTION TO HANDLING	1	1	1	1	1	1	1	1
REARS	0	1	1	0	0	2	0	0
FECAL BOLUSES	4	0	0	0	6	1	3	0
URINE POOLS	0	2	1	1	2	3	7	1
LEVEL OF AROUSAL	3	3	3	2	3	3	3	3
BEHAVIORAL OR POSTURAL ALTERATIONS d	1	1	1	1	1	1	1	1
GAIT PATTERN	1	1	1	1	1	1	1	1
SEVERITY OF GAIT ABNORMALITY	1	1	1	1	1	1	1	1
PALPEBRAL CLOSURE	1	2	2	3	1	1	1	1
PROMINENCE OF THE EYE	1	1	1	1	1	1	1	1
LACRIMATION	1	1	1	1	1	1	1	1
SALIVATION	1	1	1	1	1	4	1	2
PILOERECTION	0	0	0	0	0	0	0	0
RESPIRATION	0	0	0	1	0	0	0	0
APPEARANCE	1	1	1	1	1	1	1	1
REACTION TO VISUAL STIMULUS	1	2	2	1	2	2	1	2
REACTION TO TACTILE STIMULUS	2	1	1	1	2	2	2	2
REACTION TO AUDITORY STIMULUS	3	3	3	3	3	3	3	3
REACTION TO TAIL PINCH	4	2	3	2	2	1	4	2
VISUAL PLACING RESPONSE	1	1	1	1	1	1	1	1
AIR RIGHTING RESPONSE	1	1	1	2	1	1	1	1
PUPILLARY RESPONSE	1	1	1	1	1	1	1	1e
FORELIMB GRIP STRENGTH (G) #1	575	700	825	775	500	775	825	1100
FORELIMB GRIP STRENGTH (G) #2	500	600	650	650	f	775	700	850
HINDLIMB GRIP STRENGTH (G) #1	850	825	400	850	550	950	500	450
HINDLIMB GRIP STRENGTH (G) #2	925	650	600	775	575	950	675	600
LANDING FOOT SPY (CM) #1	8.6	5.5	6.8	6.5	6.2	7.6	7.3	7.9
LANDING FOOT SPY (CM) #2	8.0	7.5	7.0	8.2	5.7	8.3	5.5	7.3
BODY WEIGHT	479	491	530	443	479	518	479	433

#1 = FIRST MEASUREMENT

#2 = SECOND MEASUREMENT

a. The examination was conducted approximately 5.5 hours postdosage in Group II and approximately 2 hours postdosage in Group VII.

b. Dosage occurred once.

c. Observation made in the home cage.

d. Observation made in the open field.

e. Miosis identified.

f. Value was not recorded.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 4 (PAGE 1): FUNCTIONAL OBSERVATIONAL BATTERY - INDIVIDUAL DATA - FEMALE RATS

TEST SESSION 2a

RAT #	9176	9189	9190	9197	9179	9182	9192	9195
DOSAGE GROUP b	II	II	II	II	VII	VII	VII	VII
HOME CAGE BEHAVIOR	2	1	2	2	1	3	3	2
BEHAVIORAL OR POSTURAL ALTERATIONS c	1	1	1	1	1	1	1	1
REACTION TO REMOVAL	1	1	3	1	1	3	1	3
REACTION TO HANDLING	1	1	1	1	1	3	1	3
REARS	1	0	0	0	7	1	3	7
FECAL BOLUSES	0	0	5	0	0	2	0	0
URINE POOLS	0	1	2	1	1	2	0	0
LEVEL OF AROUSAL	3	3	2	2	3	3	3	3
BEHAVIORAL OR POSTURAL ALTERATIONS d	1	5	1	1	1	1	1	1
GAIT PATTERN	1	1	1	1	1	1	1	1
SEVERITY OF GAIT ABNORMALITY	1	1	1	1	1	1	1	1
PALPEBRAL CLOSURE	1	1	2	2	1	1	1	1
PROMINENCE OF THE EYE	1	1	1	1	1	2	1	1
LACRIMATION	1	1	1	1	1	1	1	1
SALIVATION	1	1	1	1	1	1	1	1
PILOERECTION	0	0	0	0	0	0	0	0
RESPIRATION	0	0	0	1	0	0	0	0
APPEARANCE	1	1	1	1	1	1	1	1
REACTION TO VISUAL STIMULUS	1	2	1	3	2	1	2	1
REACTION TO TACTILE STIMULUS	2	1	2	2	2	2	1	2
REACTION TO AUDITORY STIMULUS	3	3	3	3	3	3	4	3
REACTION TO TAIL PINCH	4	2	1	2	4	2	2	2
VISUAL PLACING RESPONSE	1	1	1	1	1	1	1	1
AIR RIGHTING RESPONSE	1	1	1	1	1	1	1	1
PUPILLARY RESPONSE	1	1	1	1	1	1	1	1
FORELIMB GRIP STRENGTH (G) #1	550	450	525	1300	500	450	925	725
FORELIMB GRIP STRENGTH (G) #2	350	425	475	950	650	225	700	850
HINDLIMB GRIP STRENGTH (G) #1	550	500	775	600	450	475	750	475
HINDLIMB GRIP STRENGTH (G) #2	450	450	575	525	425	425	725	350
LANDING FOOT SPY (CM) #1	4.6	7.9	7.4	7.6	10.2	6.2	6.0	6.5
LANDING FOOT SPY (CM) #2	7.0	6.0	5.8	8.0	10.5	8.1	6.3	7.0
BODY WEIGHT	274	380	279	308	265	270	359	290

#1 - FIRST MEASUREMENT

#2 - SECOND MEASUREMENT

a. The examination was conducted approximately 5.5 hours postdosage in Group II and approximately 2 hours postdosage in Group VII.

b. Dosage occurred once.

c. Observation made in the home cage.

d. Observation made in the open field.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 5 (PAGE 1): MOTOR ACTIVITY - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP II 0 (VEHICLE, CORN OIL) MG/KG/DAY a				
RAT #	19765	19767	19777	19785
<u>TEST SESSION 2b</u>				
NUMBER OF MOVEMENTS				
BLOCK 1	100	91	82	94
BLOCK 2	85	94	73	85
BLOCK 3	86	84	35	75
BLOCK 4	71	71	0	78
BLOCK 5	62	74	0	56
BLOCK 6	53	65	0	17
BLOCK 7	10	18	0	13
BLOCK 8	24	5	1	6
BLOCK 9	23	0	2	2
BLOCK 10	0	5	4	1
BLOCK 11	0	0	1	1
BLOCK 12	0	1	0	1
BLOCK 13	0	0	0	0
BLOCK 14	0	0	0	0
BLOCK 15	0	2	4	1
BLOCK 16	3	3	0	0
BLOCK 17	2	1	9	0
BLOCK 18	0	2	24	0
TOTAL	519	516	235	430

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred once.

b. The test was conducted approximately 5.5 hours postdosage, after functional observational battery on the day of dosage.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Crl:CD(TM)BR VAF/Plus(TM) RATS

TABLE 5 (PAGE 2): MOTOR ACTIVITY - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP II 0 (VEHICLE, CORN OIL) MG/KG/DAY a				
RAT #	19765	19767	19777	19785
<u>TEST SESSION 2b</u>				
TIME (SECONDS) SPENT IN MOVEMENT				
BLOCK 1	207	212	183	209
BLOCK 2	238	213	130	170
BLOCK 3	206	213	81	166
BLOCK 4	165	163	0	170
BLOCK 5	114	154	0	127
BLOCK 6	137	112	0	40
BLOCK 7	16	41	0	19
BLOCK 8	49	7	1	6
BLOCK 9	46	0	1	1
BLOCK 10	0	9	4	1
BLOCK 11	0	0	0	1
BLOCK 12	0	1	0	0
BLOCK 13	0	0	0	0
BLOCK 14	0	0	0	0
BLOCK 15	0	2	3	8
BLOCK 16	3	3	0	0
BLOCK 17	1	2	16	0
BLOCK 18	0	2	31	0
TOTAL	1182	1134	450	918

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred once.

b. The test was conducted approximately 5.5 hours postdosage, after functional observational battery on the day of dosage.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 6 (PAGE 1): MOTOR ACTIVITY - INDIVIDUAL DATA - FEMALE RATS

DOSAGE GROUP II 0 (VEHICLE, CORN OIL) MG/KG/DAY a				
RAT #	9176	9189	9190	9197
<u>TEST SESSION 2b</u>				
NUMBER OF MOVEMENTS				
BLOCK 1	93	62	81	89
BLOCK 2	99	71	61	92
BLOCK 3	49	65	54	41
BLOCK 4	6	44	50	89
BLOCK 5	1	24	36	52
BLOCK 6	1	5	12	20
BLOCK 7	0	0	25	26
BLOCK 8	23	0	33	20
BLOCK 9	38	0	11	8
BLOCK 10	4	2	0	3
BLOCK 11	2	1	0	2
BLOCK 12	5	0	0	3
BLOCK 13	0	29	0	2
BLOCK 14	0	7	0	7
BLOCK 15	0	0	1	0
BLOCK 16	4	1	1	3
BLOCK 17	67	0	2	4
BLOCK 18	23	0	0	4
TOTAL	415	311	367	463

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred once.

b. The test was conducted approximately 5.5 hours postdosage, after functional observational battery on the day of dosage.



PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 6 (PAGE 2): MOTOR ACTIVITY - INDIVIDUAL DATA - FEMALE RATS

DOSAGE GROUP II 0 (VEHICLE, CORN OIL) MG/KG/DAY a				
RAT #	9176	9189	9190	9197
<u>TEST SESSION 2b</u>				
TIME (SECONDS) SPENT IN MOVEMENT				
BLOCK 1	259	166	179	225
BLOCK 2	225	167	135	203
BLOCK 3	96	164	122	77
BLOCK 4	7	85	77	210
BLOCK 5	0	57	59	107
BLOCK 6	1	7	15	29
BLOCK 7	0	0	76	50
BLOCK 8	49	0	94	26
BLOCK 9	96	0	11	12
BLOCK 10	0	0	0	3
BLOCK 11	2	1	0	2
BLOCK 12	6	0	0	3
BLOCK 13	0	53	0	1
BLOCK 14	0	16	0	7
BLOCK 15	0	0	2	0
BLOCK 16	6	1	1	4
BLOCK 17	129	0	4	3
BLOCK 18	33	0	0	5
TOTAL	909	717	775	967

TOTAL = SUM OF BLOCKS; EACH BLOCK CONSISTS OF A 5 MINUTE PERIOD.

a. Dosage occurred once.

b. The test was conducted approximately 5.5 hours postdosage, after functional observational battery on the day of dosage.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - MALE RATS

DOSAGE GROUP		II	VII
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	200
		CORN OIL a	TRIADIMEFON a
RATS EXAMINED	N	4	4
ALL TISSUES APPEARED NORMAL		4	4

a. Dosage was administered on day 1 of the study.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 7 (PAGE 1): NECROPSY OBSERVATIONS - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RAT NUMBER	DAYS ON STUDY	NUMBER OF DOSAGES	OBSERVATIONS
II				
0 (VEHICLE, CORN OIL) a	19765	15	1	ALL TISSUES APPEARED NORMAL.
	19767	13	1	ALL TISSUES APPEARED NORMAL.
	19777	13	1	ALL TISSUES APPEARED NORMAL.
	19785	15	1	ALL TISSUES APPEARED NORMAL.
VII				
200 (TRIADIMEFON) a	19763	8	1	ALL TISSUES APPEARED NORMAL.
	19766	8	1	ALL TISSUES APPEARED NORMAL.
	19772	6	1	ALL TISSUES APPEARED NORMAL.
	19774	7	1	ALL TISSUES APPEARED NORMAL.

a. Dosage was administered on day 1 of the study.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)		II 0(VEHICLE) CORN OIL a	VII 200 TRIADIMEFON a
RATS EXAMINED	N	4	4
ALL TISSUES APPEARED NORMAL		4	4

a. Dosage was administered on day 1 of the study.

PROTOCOL 012-014: NEUROTOXICITY EVALUATION OF POSITIVE CONTROL SUBSTANCES IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 7 (PAGE 1): NECROPSY OBSERVATIONS - INDIVIDUAL DATA - FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RAT NUMBER	DAYS ON STUDY	NUMBER OF DOSAGES	OBSERVATIONS
II				
0 (VEHICLE, CORN OIL) a	9176	13	1	ALL TISSUES APPEARED NORMAL.
	9189	15	1	ALL TISSUES APPEARED NORMAL.
	9190	15	1	ALL TISSUES APPEARED NORMAL.
	9197	13	1	ALL TISSUES APPEARED NORMAL.
VII				
200 (TRIADIMEFON) a	9179	7	1	ALL TISSUES APPEARED NORMAL.
	9182	8	1	ALL TISSUES APPEARED NORMAL.
	9192	8	1	ALL TISSUES APPEARED NORMAL.
	9195	7	1	ALL TISSUES APPEARED NORMAL.

a. Dosage was administered on day 1 of the study.

PROTOCOL 012-015: NEUROTOXICITY EVALUATION OF DDT IN Crl:CD®BR  
VAF/Plus® RATS

A. Methodology

1. General Experimental Design and Procedures

The purpose of this study was to evaluate DDT using a functional observational battery (FOB). Data obtained in this study are intended for use as positive control information for validation of the Test Facility's functional observational battery, and for certification of technicians conducting the testing, in accordance with the testing guidelines of the U.S. Environmental Protection Agency (EPA). The study was conducted in compliance with EPA Good Laboratory Practices (GLPs).

Each group was randomly assigned four Crl:CD®BR VAF/Plus® (Sprague-Dawley) male and female rats. DDT<sup>a</sup> was administered orally via gavage once to these rats. Dosages of 0 (Vehicle, corn oil) and 75 mg/kg/day of DDT were administered to the rats at concentrations of 0 and 75 mg/mL, respectively. The vehicle was Mazola® corn oil (Best Foods). The dosage volume was 1 mL/kg. In a previous study conducted at the Test Facility (Argus Research Laboratories, Inc., Protocol Number: 012-014) the dosage volume for DDT was 5 mL/kg and only minimal effects were observed. Preliminary results indicated that the dosage volume was a factor, so the dosage volume was decreased for this test.

- 
- a. All dosage concentrations were prepared based on the 100% activity of the test substance.

The rats were examined at least twice daily for viability during the study. The rats were also examined for clinical observations and general health at least weekly during the acclimation period, before test substance administration on the day of dosage, daily for the two days after dosage and weekly until scheduled sacrifice. Body weights for the rats were recorded at least weekly during the acclimation and on the day the Functional Observational Battery (FOB) was conducted for each rat, before test substance administration on the day of dosage, daily for the two days after dosage and weekly until scheduled sacrifice. Feed consumption values were recorded on the day of test substance administration and for two days after the test substance was administered. The FOB evaluations were conducted once after dosage administration.

Rats that were found dead were necropsied on the day the event occurred using the procedures described below. All surviving rats were sacrificed by carbon dioxide asphyxiation and necropsied. Tissues with gross lesions were preserved in neutral buffered 10% formalin for possible future analysis. The carcasses were discarded.

## 2. Conduct of Study

The in-life phase of the study occurred as follows:

<u>Phase</u>	<u>Dates</u>
Arrival Date	03/10/92
Administration Period	03/25/92
Functional Observational Battery (FOB)	03/25/92
Scheduled Sacrifice	06/11/92

The standards and/or requirements of the U.S. Environmental Protection Agency (EPA) were used as the basis for study design and compliance with Good Laboratory Practices (GLPs).

3. Sponsor

This study was sponsored by Argus Research Laboratories, Inc., 905 Sheehy Drive, Horsham, Pennsylvania 19044. The Sponsor's Representative was Alan M. Hoberman, Ph.D., D.A.B.T. (Director of Research).

4. Test Facility, Study Director and Participating Personnel

The study was conducted by and at Argus Research Laboratories, Inc., 905 Sheehy Drive, Horsham, Pennsylvania 19044. The Study Director was John A. Foss, Ph.D. (Group Leader, Neurotoxicology).

Key personnel participating in the conduct of the study were John F. Barnett, B.S. (Director of Laboratory Operations), Ronald J. McCarty, B.S. (Assistant Director of Laboratory Operations), Danielle R. Stackhouse, B.S. (Laboratory Associate/Assistant Supervisor), Barbara J. Le May, B.A., Jacqueline Manigault, B.S., Elizabeth A. Jenkins, B.S., Susan L. DeHaven, B.S. (Laboratory Associates), Karen S. Willey (Animal Care Technician), Anthony S. Conlon, B.S. (Information Specialist/Trainer), Cheryl L. Van, B.A. (Methods Writer) and Georgia Y. Burnett, A.A.S. (Word Processor). Additional personnel participating in the conduct of the study are identified in the raw data.

Curricula vitae and training records of the personnel involved in the conduct of the study are on file at Argus Research Laboratories, Inc.

5. Test Substance and Vehicle Identification

The test substance, DDT (Lot 40H3406)<sup>a</sup>, a white powder was obtained from Sigma Chemical Company. It was received at the Test Facility on May 29,

- 
- a. All dosage concentrations were prepared based on the 100% activity of the test substance.



1991 and was stored refrigerated. Characterization and analyses for purity and stability of the test substance is maintained by the Supplier. The vehicle, Mazola® corn oil (Best Foods) (Lot SEP 26 92 B), a yellow liquid, was stored at room temperature.

B. Animal Data

1. Test System

The Charles River Crl:CD®BR VAF/Plus® rat was selected for evaluation of DDT because the strain has been widely used throughout industry for nonclinical studies for toxicity.

On March 10, 1992, 43 male rats and 43 female rats were received from Charles River Laboratories, Inc., Raleigh, North Carolina. These rats were approximately 51 and 50 days of age, respectively, at receipt (approximate birthdates: January 20 and January 21, 1992, respectively). On the day after arrival, the male rats weighed from 208 to 248 g, and the female rats weighed from 140 to 187 g.

2. Experimental Design and Control of Bias

Upon arrival, each rat was assigned to individual housing on the basis of a computer-generated randomization procedure. Each male rat and female rat was assigned a temporary pretest number (1 through 43), which was used for identification during the acclimation period. Unique permanent numbers were assigned on the day the rats were placed on study. Each rat was then individually identified with a Monel® self-piercing ear tag<sup>a</sup> inscribed with the rat's designated unique number.

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a. Gey Band and Tag Co., Inc., No. MSPT 20101.

PROTOCOL 012-015: NEUROTOXICITY EVALUATION OF DDT IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 1): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY - MALE AND FEMALE RATS

DOSAGE GROUP		I	II
DOSAGE (MG/KG/DAY) <sup>a</sup>		0 (VEHICLE)	75
RATS TESTED	N	8	8
HOME CAGE BEHAVIOR			
Sleeping	N	2	0
Awake, immobile	N	5	2
Normal movement	N	1	0
Unusual posture, tonic convulsion	N	0	0
Unusual behavior	N	0	6**
BEHAVIORAL OR POSTURAL ALTERATIONS <sup>b</sup>			
None	N	8	3
Stereotyped behavior	N	0	1
Bizarre behavior	N	0	0
Twitches or tremors in limbs	N	0	1
Whole body tremors or spasms	N	0	3
Unusual posture	N	0	0
Tonic-clonic seizure	N	0	0
REACTION TO REMOVAL			
(1) Easily removed	N	8	6
(2) Vocalization, no resistance	N	0	0
(3) Runs or freezes (follows hand)	N	0	2
(4) Tail or throat rattles	N	0	0
MEAN SCORE		1.0	1.5
REACTION TO HANDLING			
(1) Quiet, no resistance	N	8	5
(2) Vocalization, no resistance	N	0	0
(3) Tense	N	0	3
(4) Squirming	N	0	0
MEAN SCORE		1.0	1.8
REARS (MALE)	MEAN ± S.D.	4.8 ± 3.4	0.0 ± 0.0*
REARS (FEMALE)	MEAN ± S.D.	3.0 ± 3.2	1.7 ± 2.1
FECAL BOLUSES (MALE)	MEAN ± S.D.	1.0 ± 1.2	0.0 ± 0.0
FECAL BOLUSES (FEMALE)	MEAN ± S.D.	0.2 ± 0.5	0.5 ± 1.0

(n) = Score assigned to graded test items.

a. Dosage occurred on day 1 of the study.

b. Observation made at the home cage.

\* Significantly different from the vehicle control group value ( $P \leq 0.05$ ).

\*\* Significantly different from the vehicle control group value ( $P \leq 0.01$ ).

PROTOCOL 012-015: NEUROTOXICITY EVALUATION OF DDT IN Crl:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 2): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY - MALE AND FEMALE RATS

DOSAGE GROUP		I	II
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE)	75
RATS TESTED	N	8	8
URINE POOLS (MALE)	MEAN $\pm$ S.D.	1.0 $\pm$ 1.4	0.2 $\pm$ 0.5
URINE POOLS (FEMALE)	MEAN $\pm$ S.D.	0.2 $\pm$ 0.5	0.5 $\pm$ 1.0
LEVEL OF AROUSAL			
(1) Very low (stuporous)	N	0	0
(2) Low (sluggish)	N	3	0
(3) Apparently normal	N	5	4
(4) High (sudden startle)	N	0	4
(5) Very high (sudden running, vocalization)	N	0	0
	MEAN SCORE	2.6	3.5**
BEHAVIORAL OR POSTURAL ALTERATIONS <sup>b</sup>			
None	N	8	0**
Stereotyped behavior	N	0	0
Bizarre behavior	N	0	0
Twitches or tremors in limbs	N	0	0
Whole body tremors or spasms	N	0	8**
Unusual posture	N	0	0
Tonic-clonic seizure	N	0	0
GAIT PATTERN			
Apparently normal	N	8	8
Ataxic	N	0	0
Exaggerated movements (limbs splayed)	N	0	0
Spastic, tip-toe	N	0	0
Duck-walk	N	0	0
Scissors gait	N	0	0
SEVERITY OF GAIT ABNORMALITY			
(1) Normal	N	4	4
(2) Slight	N	0	0
(3) Moderate	N	0	0
(4) Severe	N	0	0
	MEAN SCORE	1.0	1.0

(n) = Score assigned to graded test items.

a. Dosage occurred on day 1 of the study.

b. Observation made in the open field.

\*\* Significantly different from the vehicle control group value ( $P \leq 0.01$ ).

PROTOCOL 012-015: NEUROTOXICITY EVALUATION OF DDT IN Crl:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 3): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY - MALE AND FEMALE RATS

DOSAGE GROUP		I	II
DOSAGE (MG/KG/DAY) <sup>a</sup>		0 (VEHICLE)	75
RATS TESTED	N	8	8
PALPEBRAL CLOSURE			
(1) Wide open	N	6	6
(2) Slightly drooping	N	2	2
(3) Half closed	N	0	0
(4) Completely shut	N	0	0
MEAN SCORE		1.2	1.2
PROMINENCE OF THE EYE			
Normal	N	8	8
Exophthalmos	N	0	0
Enophthalmos	N	0	0
LACRIMATION			
(1) No excess	N	8	8
(2) Excess at margin	N	0	0
(3) Persistent dampness	N	0	0
(4) Extends beyond margin	N	0	0
MEAN SCORE		1.0	1.0
SALIVATION			
(1) No excess	N	8	8
(2) Slight wetness at margin	N	0	0
(3) Wetness 1/4 to 1/2 submaxillary	N	0	0
(4) Wetness over entire submaxillary	N	0	0
MEAN SCORE		1.0	1.0
PILOERECTION PRESENT	N	0	0
RESPIRATION ABNORMAL	N	0	0

(n) = Score assigned to graded test item.

a. Dosage occurred on day 1 of the study.

PROTOCOL 012-015: NEUROTOXICITY EVALUATION OF DDT IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 4): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY - MALE AND FEMALE RATS

DOSAGE GROUP		I	II
DOSAGE (MG/KG/DAY) <sup>a</sup>		0(VEHICLE)	75
RATS TESTED	N	8	8
APPEARANCE			
(1) Clean and groomed	N	8	8
(2) Unkempt	N	0	0
(3) Stained by urine or feces	N	0	0
MEAN SCORE		1.0	1.0
REACTION TO VISUAL STIMULUS			
(1) None	N	5	5
(2) Orienting	N	3	1
(3) Startle	N	0	2
(4) More energetic reaction	N	0	0
(5) Attacks	N	0	0
MEAN SCORE		1.4	1.6
REACTION TO TACTILE STIMULUS			
(1) None	N	1	5
(2) Orienting	N	7	3
(3) Startle	N	0	0
(4) More energetic reaction	N	0	0
(5) Attacks	N	0	0
MEAN SCORE		1.9	1.8 <sup>a</sup>
REACTION TO AUDITORY STIMULUS			
(1) None	N	0	1
(2) Orienting	N	3	3
(3) Startle	N	5	4
(4) More energetic reaction	N	0	0
(5) Attacks	N	0	0
MEAN SCORE		2.6	2.4

(n) = Score assigned to graded test items.

a. Dosage occurred on day 1 of the study.

\* Significantly different from the vehicle control group value ( $P \leq 0.05$ ).

PROTOCOL 012-015: NEUROTOXICITY EVALUATION OF DDT IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 1 (PAGE 5): FUNCTIONAL OBSERVATIONAL BATTERY - SUMMARY - MALE AND FEMALE RATS

DOSAGE GROUP		I	II
DOSAGE (MG/KG/DAY) <sup>a</sup>		0 (VEHICLE)	75
RATS TESTED	N	8	8
REACTION TO TAIL PINCH			
(1) None	N	0	8
(2) Orienting	N	7	0
(3) Startle	N	1	0
(4) More energetic reaction	N	0	0
(5) Attacks	N	0	0
MEAN SCORE		2.1	1.0
VISUAL PLACING RESPONSE			
(1) Early extension	N	8	7
(2) Extension after contact	N	0	0
(3) No extension	N	0	1
MEAN SCORE		1.0	1.4
AIR RIGHTING RESPONSE			
(1) Lands with all feet on the ground	N	8	5
(2) Uncoordinated landing	N	0	2
(3) Lands on back	N	0	0
MEAN SCORE		1.0	1.3 <sup>b</sup>
NO PUPILLARY RESPONSE	N	0	0
GRIP-STRENGTH FORELIMB (G)			
Maximum (MALE)	MEAN ± S.D.	662 ± 92	500 ± 117
Maximum (FEMALE)	MEAN ± S.D.	656 ± 160	631 ± 62
Average (MALE)	MEAN ± S.D.	641 ± 107	463 ± 89 <sup>a</sup>
Average (FEMALE)	MEAN ± S.D.	591 ± 119	572 ± 34
GRIP-STRENGTH HINDLIMB (G)			
Maximum (MALE)	MEAN ± S.D.	556 ± 85	467 ± 170
Maximum (FEMALE)	MEAN ± S.D.	581 ± 120	594 ± 103
Average (MALE)	MEAN ± S.D.	516 ± 77	421 ± 146
Average (FEMALE)	MEAN ± S.D.	531 ± 85	544 ± 98
LANDING FOOT SPREAD AVERAGE (CM)	MEAN ± S.D.	8.2 ± 2.0	6.0 ± 1.6 <sup>a</sup>
			[ 7 ] <sup>b</sup>
BODY WEIGHT (MALE)	MEAN ± S.D.	348 ± 22	346 ± 35
BODY WEIGHT (FEMALE)	MEAN ± S.D.	227 ± 10	229 ± 18

(n) = Score assigned to graded test items.

[ ] = Number of values averaged.

a. Dosage occurred on day 1 of the study.

b. Excludes a value that was not recorded.

\* Significantly different from the vehicle control group value (P<0.05).

PROTOCOL 012-015: NEUROTOXICITY EVALUATION OF DDT IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 1): FUNCTIONAL OBSERVATIONAL BATTERY - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY) <sup>a</sup>	I 0(VEHICLE)				II 75			
RAT #	11016	11017	11020	11022	11018	11019	11021	11023
HOME CAGE BEHAVIOR	2	1	2	3	5	5	5	5
BEHAVIORAL OR POSTURAL ALTERATION <sup>b</sup>	1	1	1	1	1	5	1	4
REACTION TO REMOVAL	1	1	1	1	1	1	1	1
REACTION TO HANDLING	1	6	1	1	1	3	1	1
REARS	0	0	5	8	0	0	0	0
FECAL BOLUSES	2	0	0	2	0	0	0	0
URINE POOLS	0	3	1	3	0	1	0	0
LEVEL OF AROUSAL	3	1	3	3	4	4	4	4
BEHAVIORAL OR POSTURAL ALTERATION <sup>c</sup>	1	1	1	1	5	5	5	5
GAIT PATTERN	1	1	1	1	1	1	1	1
SEVERITY OF GAIT ABNORMALITY	1	2	1	1	1	1	1	1
PALPEBRAL CLOSURE	1	1	1	1	1	2	1	1
PROMINENCE OF THE EYE	1	1	1	1	1	1	1	1
LACRIMATION	1	1	1	1	1	1	1	1
SALIVATION	1	1	1	1	1	1	1	1
PILOERECTION	0	0	0	0	0	0	0	0
RESPIRATION	0	1	0	0	0	0	0	0
APPEARANCE	1	2	1	1	1	1	1	1
REACTION TO VISUAL STIMULUS	1	2	1	2	1	1	1	3
REACTION TO TACTILE STIMULUS	2	3	2	2	2	1	1	1
REACTION TO AUDITORY STIMULUS	3	2	2	2	2	1	3	3
REACTION TO TAIL PINCH	2	2	2	2	1	1	1	1
VISUAL PLACING RESPONSE	1	1	1	1	1	3	1	1
AIR RIGHTING RESPONSE	1	1	1	1	1	d	2	1
PUPILLARY RESPONSE	1	1	1	1	1	1	1	1
FORELIMB GRIP STRENGTH (G) #1	625	550	575	800	625	400	550	400
FORELIMB GRIP STRENGTH (G) #2	550	600	625	800	400	375	575	375
HINDLIMB GRIP STRENGTH (G) #1	500	450	550	675	375	275	d	600
HINDLIMB GRIP STRENGTH (G) #2	525	475	375	575	525	250	d	500
LANDING FOOT SPLAY (CM) #1	9.7	8.8	5.5	7.5	5.5	d	6.5	6.4
LANDING FOOT SPLAY (CM) #2	10.8	8.8	5.5	7.8	6.0	d	9.5	7.8
BODY WEIGHT	325	361	334	372	398	328	335	323

VALUES ARE THE QUANTITY, CATEGORY NUMBER, QUANTAL RESPONSE OR SCORE RECORDED FOR EACH ITEM IN THE BATTERY.

#1 - FIRST MEASUREMENT

#2 - SECOND MEASUREMENT

a. Dosage occurred on day 1 of the study.

b. Observation made at the home cage.

c. Observation made in the open field.

d. Value was not recorded.

PROTOCOL 012-015: NEUROTOXICITY EVALUATION OF DDT IN Crl:CD(TM)BR VAF/Plus(TM) RATS

TABLE 3 (PAGE 1): FUNCTIONAL OBSERVATIONAL BATTERY - INDIVIDUAL DATA - FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY) <sup>a</sup>	I 0 (VEHICLE)				II 75			
RAT #	11025	11029	11030	11031	11024	11026	11027	11028
HOME CAGE BEHAVIOR	2	1	2	2	5	2	5	2
BEHAVIORAL OR POSTURAL ALTERATION <sup>b</sup>	1	1	1	1	5	1	5	2
REACTION TO REMOVAL	1	1	1	1	3	3	1	1
REACTION TO HANDLING	1	1	1	1	1	3	3	1
REARS	7	1	4	0	1	4	d	0
FECAL BOLUSES	1	0	0	0	0	0	2	0
URINE POOLS	0	0	0	1	0	0	2	0
LEVEL OF AROUSAL	3	2	3	3	3	3	4	4
BEHAVIORAL OR POSTURAL ALTERATION <sup>c</sup>	1	1	1	1	5	5	5	5
GAIT PATTERN	1	1	1	1	1	1	1	1
SEVERITY OF GAIT ABNORMALITY	1	1	1	1	1	1	1	1
PALPEBRAL CLOSURE	1	2	1	1	1	1	1	1
PROMINENCE OF THE EYE	1	1	1	1	1	1	1	1
LACRIMATION	1	1	1	1	1	1	1	1
SALIVATION	1	1	1	1	1	1	1	1
PILORECTION	0	0	0	0	0	0	0	0
RESPIRATION	0	0	0	0	0	0	0	0
APPEARANCE	1	1	1	1	1	1	1	1
REACTION TO VISUAL STIMULUS	2	1	1	1	3	2	1	1
REACTION TO TACTILE STIMULUS	2	2	2	1	1	2	1	1
REACTION TO AUDITORY STIMULUS	3	2	3	3	3	3	3	2
REACTION TO TAIL PINCH	3	2	2	2	1	1	1	1
VISUAL PLACING RESPONSE	1	1	1	1	1	1	1	1
AIR RIGHTING RESPONSE	1	1	1	1	2	1	1	1
PUPILLARY RESPONSE	1	1	1	1	1	0	1	1
FORELIMB GRIP STRENGTH (G) #1	425	750	775	675	625	550	575	700
FORELIMB GRIP STRENGTH (G) #2	400	525	525	650	450	550	650	475
HINDLIMB GRIP STRENGTH (G) #1	425	550	675	450	725	425	525	450
HINDLIMB GRIP STRENGTH (G) #2	400	525	550	675	650	625	450	500
LANDING FOOT SPREAD (CM) #1	5.0	10.0	10.2	9.5	6.4	6.0	2.5	5.0
LANDING FOOT SPREAD (CM) #2	4.6	8.9	9.5	9.2	7.4	7.0	4.5	3.5
BODY WEIGHT	219	218	237	235	255	218	222	220

VALUES ARE THE QUANTITY, CATEGORY NUMBER, QUANTAL RESPONSE OR SCORE RECORDED FOR EACH ITEM IN THE BATTERY.

#1 = FIRST MEASUREMENT

#2 = SECOND MEASUREMENT

a. Dosage occurred on day 1 of the study.

b. Observation made at the home cage.

c. Observation made in the open field

d. Value was not recorded.



PROTOCOL 012-015: NEUROTOXICITY EVALUATION OF DDT IN Crl:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - MALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY) <sup>a</sup>		I 0 (VEHICLE)	II 75
RATS EXAMINED <sup>b</sup>	N	4	4
FOUND DEAD	N	0	2 <sup>c,d</sup>
LUNGS: MOTTLED DARK RED TO BLACK	N	0	1 <sup>c</sup>

a. Dosage occurred on day 1 of the study.

b. Refer to the individual clinical observations table (Table 6) for external observations confirmed at necropsy.

c. Occurred for rat 11019, which was found dead on day 1 of the study.

d. Occurred for rat 11021, which was found dead on day 1 of the study.

PROTOCOL 012-015: NEUROTOXICITY EVALUATION OF DDT IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 7 (PAGE 1): NECROPSY OBSERVATIONS - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY) <sup>a</sup>	RAT NUMBER	DAY OF NECROPSY	DOSAGES ADMINISTERED	OBSERVATIONS <sup>b</sup>
<b>I</b>				
0 (VEHICLE)	11016	DS 79	1	ALL TISSUES APPEARED NORMAL.
	11017	DS 79	1	ALL TISSUES APPEARED NORMAL.
	11020	DS 79	1	ALL TISSUES APPEARED NORMAL.
	11022	DS 79	1	ALL TISSUES APPEARED NORMAL.
<b>II</b>				
75	11018	DS 79	1	ALL TISSUES APPEARED NORMAL.
	11019	DS 2c	1	FOUND DEAD ON DAY 1 OF THE STUDY. LUNGS: MOTTLED, DARK RED TO BLACK. ALL OTHER TISSUES APPEARED
	11021	DS 1	1	FOUND DEAD ON DAY 1 OF THE STUDY. ALL TISSUES APPEARED NORMAL.
	11023	DS 79	1	ALL TISSUES APPEARED NORMAL.

DS - DAY OF THE STUDY

- a. Dosage occurred on day 1 of the study.
- b. Refer to the individual clinical observations table (Table 6) for external observations confirmed at necropsy.
- c. Found dead on day 1 of the study and was refrigerated until time of necropsy on day 2 of the study.

PROTOCOL 012-015: NEUROTOXICITY EVALUATION OF DDT IN Crl:CD(TM)BR VAF/Plus(TM) RATS

TABLE 2 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY) <sup>a</sup>		I 0 (VEHICLE)	II 75
RATS EXAMINED	N	4	4
MORTALITY	N	0	0
APPEARED NORMAL	N	4	4

a. Dosage occurred on day 1 of the study.

PROTOCOL 012-015: NEUROTOXICITY EVALUATION OF DDT IN Cr1:CD(TM)BR VAF/Plus(TM) RATS

TABLE 7 (PAGE 1): NECROPSY OBSERVATIONS - INDIVIDUAL DATA - FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)a	RAT NUMBER	DAY OF NECROPSY	DOSAGES ADMINISTERED	OBSERVATIONS
I				
0(VEHICLE)	11025	DS 79	1	ALL TISSUES APPEARED NORMAL.
	11029	DS 79	1	ALL TISSUES APPEARED NORMAL.
	11030	DS 79	1	ALL TISSUES APPEARED NORMAL.
	11031	DS 79	1	ALL TISSUES APPEARED NORMAL.
II				
75	11024	DS 79	1	ALL TISSUES APPEARED NORMAL.
	11026	DS 79	1	ALL TISSUES APPEARED NORMAL.
	11027	DS 79	1	ALL TISSUES APPEARED NORMAL.
	11028	DS 79	1	ALL TISSUES APPEARED NORMAL.

DS - DAY OF THE STUDY

a. Dosage occurred on day 1 of the study.

APPENDIX 8  
MOTOR ACTIVITY VALIDATION

**The Assessment of Motor Activity  
in Neonatal and Adult Rodents  
using Passive Infrared Sensors**

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Poster Presented at the

12<sup>th</sup> Annual Meeting of the American College of Toxicology

Savannah, Georgia

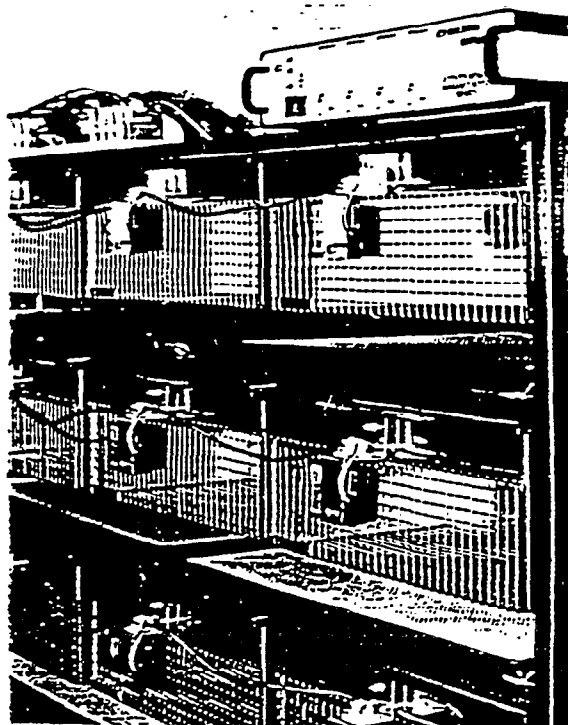
October, 1991

## ABSTRACT

Motor activity is frequently assessed in neuropharmacology and neurotoxicology studies, and the measurement of motor activity is an important component of the current EPA neurotoxicology testing guidelines. Given the widespread use of motor activity tests, there is continued interest in systems that will permit efficient measurement of movement. The data reported here describe the application of passive infrared sensors to monitor the movement of neonatal and adult rodents. By detecting changes in the temperature pattern within its field, a single sensor is able to monitor an animal's movement within a test cage. Data from multiple sensor-cage combinations can be collected in a single test session. The output of each sensor is proportional to the extent of movement, and measurements of the number of movements and the time spent in movement can be derived. Data are provided to document the ability of this system to measure the reduction in activity that occurs in a single session as adult rats and mice adapt to the test environment, and the change in activity across sessions when developing rats are examined. Dosage-dependent changes in activity are also demonstrated following acute administration of d-amphetamine or chlorpromazine. These data illustrate the utility of this system for detecting generalized changes in motor activity.

## GENERAL METHOD

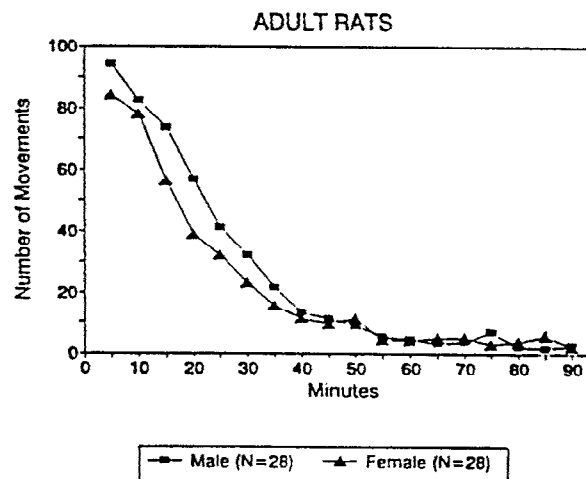
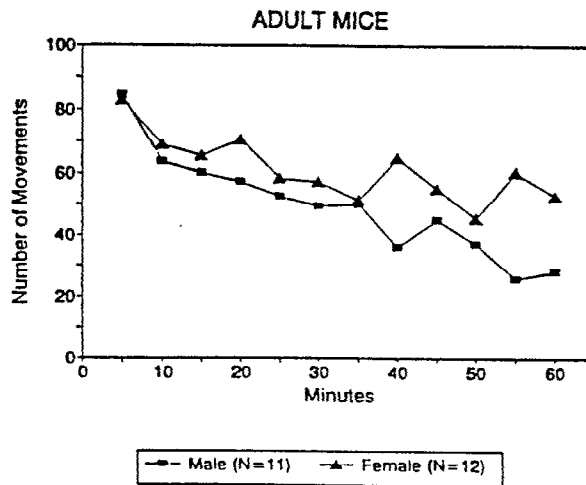
Single rats or mice were placed in double-width, stainless steel cages and monitored for one to two hour periods. Stainless steel sheets were placed over the mesh floor when mice and neonatal rats were tested. Infrared energy generated by an animal was focused onto a pyroelectric sensor by an array of Fresnel lens that collected the energy from different zones within the cage. The animal's movement through the zones induced temperature changes in the sensor, which produced proportional voltage signals. In the experiments described here, those signals were sampled at 200 ms intervals and converted to digital values. Threshold values of 0.3 and 1.0 volts delimited "large" and "small" movement categories, and a single movement was defined as a set of consecutive samples in one movement category. Both the number of "large" and "small" movements and the total time spent in these movements were tabulated at five-minute intervals. The data for the two movement categories were combined in the analysis, and only the data for the number of movements are displayed here (data for time spent in movement were similar). The data were analyzed using repeated-measures analyses of variance with sex or dosage group as between-subject factors and the set of measurement intervals in a session as a within-subject factor (the Greenhouse-Geiser correction was used in deriving probabilities for effects involving within-subject factors).



## ADULT MICE AND RATS

Adult mice (three months of age) and rats (three to five months of age) were monitored for 60 and 90 minutes periods, respectively. Movements decreased in all the groups within the test sessions [ $F(11,231)=15.6$ ,  $p \leq .001$  for the mice and  $F(17,918)=182.9$ ,  $p \leq .001$  for the rats], presumably reflecting the animals's adaptation to the novel environment, although the changes were more pronounced in the rats. The average number of movements in each interval ( $\pm$ S.D.) was 50.7 ( $\pm 18.9$ ) for the male mice and 61.7 ( $\pm 10.7$ ) for the female mice; the averages for the male and female rats were 25.8 ( $\pm 9.8$ ) and 21.8 ( $\pm 8.2$ ), respectively. These differences between males and females were not significant ( $p > .05$ ), but the differences between the sexes in the patterns across the measurement intervals were significant [ $F(11,231)=3.2$ ,  $p \leq .01$  for the mice and  $F(17,918)=2.3$ ,  $p \leq .05$  for the rats].

If the mean and standard deviation for the adult male rats are used as examples, inferences can be made regarding the power of statistical tests using tables provided by J. Cohen (Statistical Power Analysis for the Behavioral Sciences (2<sup>nd</sup> Ed.), 1987). In a design with four groups, a maximum change of one standard deviation from the control value (a 40% change), and the other groups spaced at equal intervals between the extremes, the probability of obtaining significance at  $p \leq .05$  is 50% for group sizes of 10, 70% for group sizes of 15, and 85% for group sizes of 20.



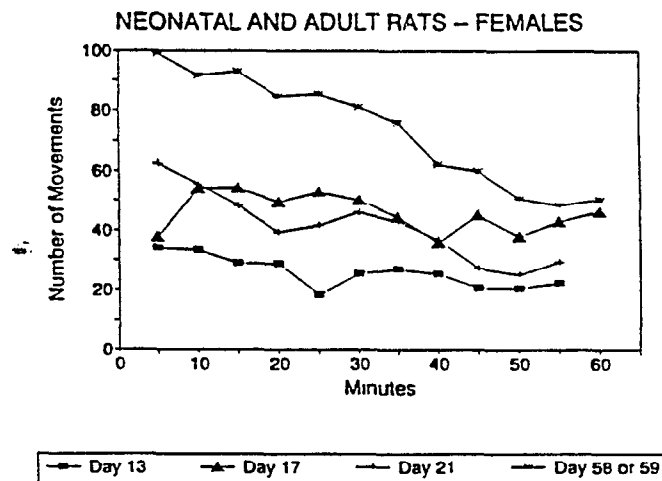
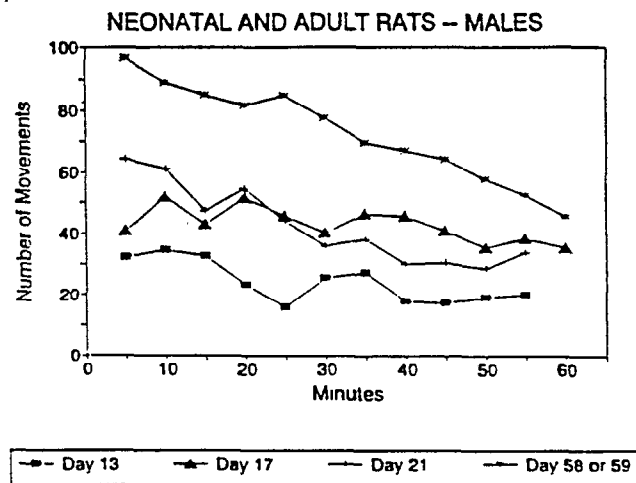


## NEONATAL AND ADULT RATS

One male and one female rat were selected from each of 22 litters and monitored in the apparatus for 60 minutes on postnatal days 13, 17 and 21, and on postnatal day 58 or 59 (postnatal day 1 was the day of birth). Differences between the values across the measurement intervals were significant at all the ages (all  $p \leq .05$ ), but the pattern on day 17 did not have a significant linear trend. The average number of movements in each interval at each age is listed in the following table.

Day	MALES		FEMALES	
	Mean	Standard Deviation	Mean	Standard Deviation
Day 13	24.0	14.3	25.9	11.8
Day 17	35.2	24.9	46.4	26.8
Day 21	42.4	19.5	41.3	18.1
Day 58/59	72.4	10.4	50.2	10.7

Neither the differences in the average values between male and female rats nor their differences in the patterns across the measurement intervals were significant (all  $p > .1$ ).

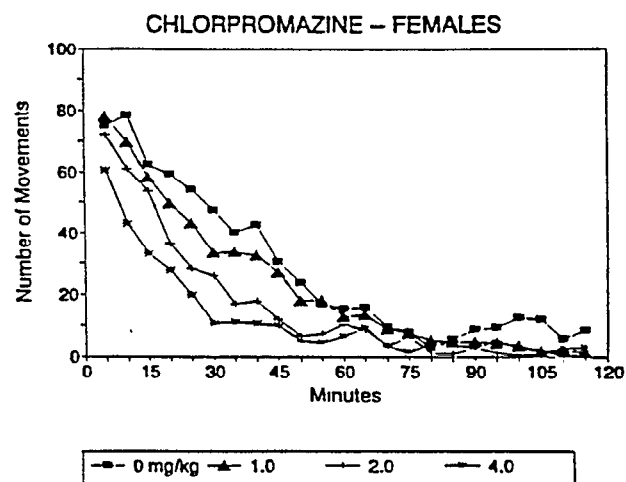
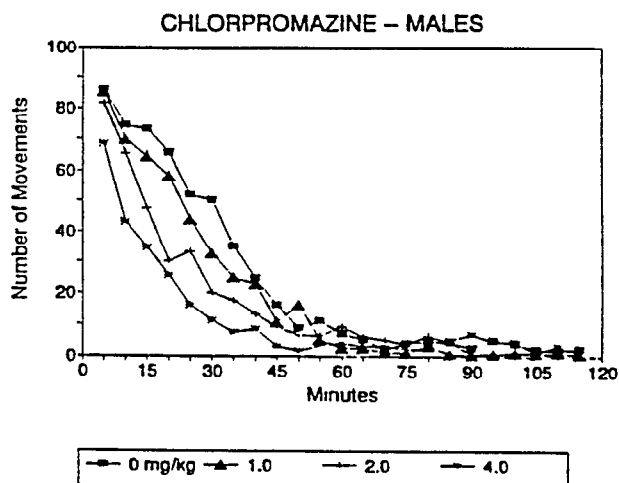
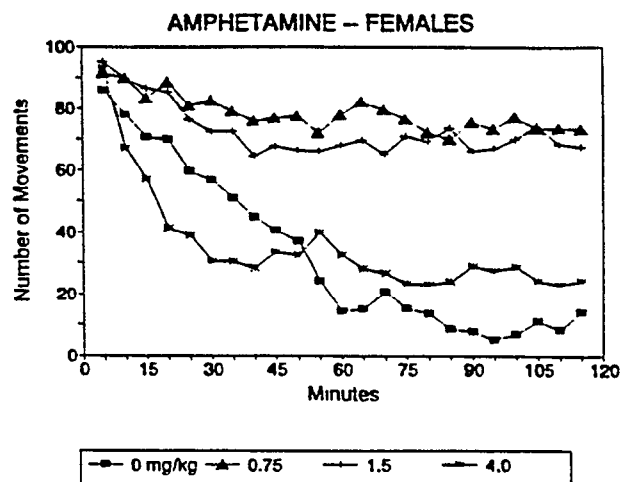
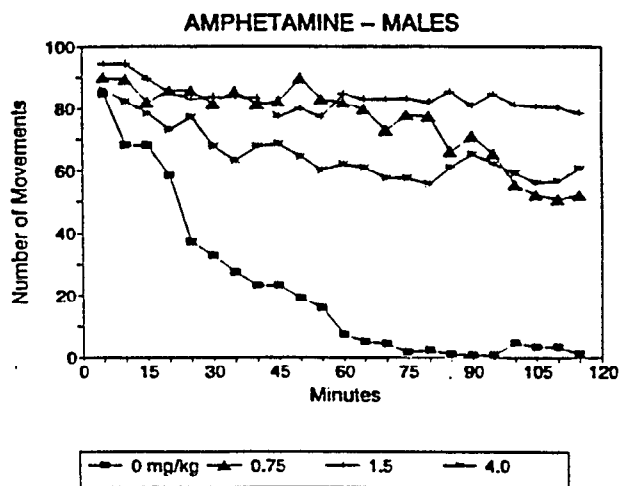


## DOSAGE RESPONSE

A group of 15 male rats and 15 female rats (approximately 60 days of age at the start of the experiment) were administered four dosages of d-amphetamine (0, 0.75, 1.5 and 4.0 mg/kg, i.p., immediately before testing) and four dosages of chlorpromazine (0, 1.0, 2.0 and 4.0 mg/kg, i.p., 70 minutes before testing). The sequences of dosages were counterbalanced across rats, and tests were conducted at least two days apart. Motor activity was monitored for at least 115 minutes in each test session. The average number of movements in each interval at each dosage is listed in the following table.

Dosage (mg/kg)	MALES		FEMALES	
	Mean	Standard Deviation	Mean	Standard Deviation
d-Amphetamine				
0	21.4	9.1	33.0	13.7
0.75	75.5	7.4	78.4	13.0
1.5	83.4	9.6	72.7	15.7
4.0	65.4	26.2	35.0	17.2
Chlorpromazine				
0	23.4	8.6	28.0	13.3
1.0	19.5	5.6	23.1	10.0
2.0	16.0	5.6	16.7	6.0
4.0	11.4	6.0	12.0	8.8

The statistical analyses were based on the data collected in the first 60 minutes. Differences among the values recorded at each dosage were significant [ $F(3,42) > 11.0$ ,  $p \leq .001$ ] for each test substance in both the male and female rats. In pairwise comparisons, the number of movements detected after the 0.75, 1.5, and 4.0 dosages of amphetamine in the male rats were significantly increased (all  $p \leq .01$ ), as compared to the vehicle control. The increase was smallest at the highest dosage, and this reduction probably reflected a change in behavior from increased ambulation to stereotypical patterns such as head bobbing. In female rats the values for the 0 and 4.0 mg/kg dosages were not significantly different ( $p > .1$ ), but significantly more movements ( $p \leq .001$ ) were detected after the 0.75 and 1.5 mg/kg dosages. Chlorpromazine reduced the number of movements in a dosage-dependent pattern, with the 2.0 and 4.0 mg/kg dosages producing significant reductions ( $p \leq .01$ ) in both male and female rats, as compared to the vehicle control.

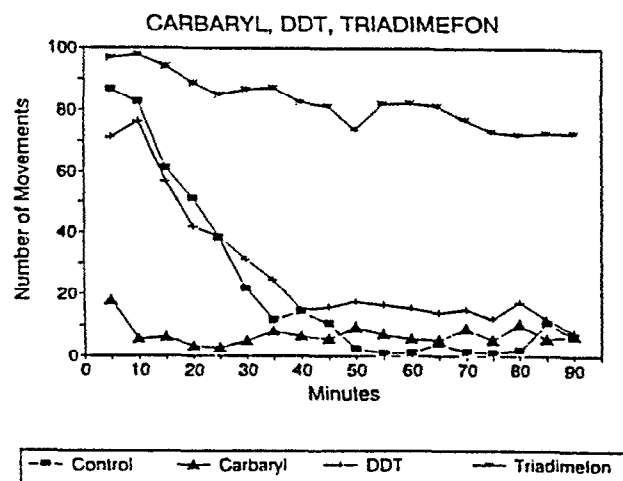
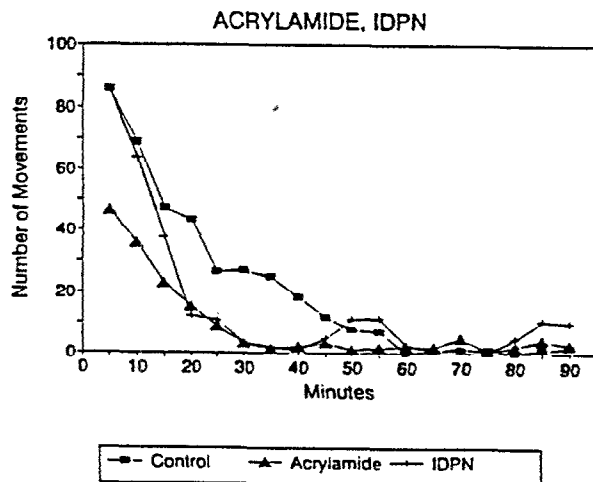


## OTHER TEST SUBSTANCES

Groups of four male and four female rats (three to five months of age) were administered acrylamide (40 mg/kg/day for nine days), 3,3-iminodipropionitrile (IDPN; 200 mg/kg/day for three days), or their vehicle intraperitoneally; or carbaryl (75 mg/kg), DDT (75 mg/kg), triadimefon (200 mg/kg), or their vehicle once orally by gavage. Motor activity was monitored for 90 minute periods 13 days following the first dosage of acrylamide and IDPN, and on the day of dosage for the other test substances (approximately 3 hours after administration of carbaryl and triadimefon and 6.5 hours after administration of DDT). The average number of movements in each interval for each test substance is listed in the following table (data for male and female rats in each group were combined).

Test Substance	Mean	Standard Deviation
<u>I.P. Administration</u>		
Control (Saline)	20.8	11.3
Acrylamide	8.8	4.4
IDPN	15.2	6.6
<u>Oral Administration</u>		
Control (Corn Oil)	22.6	5.5
Carbaryl	7.0	6.1
DDT	27.5	10.6
Triadimefon	82.5	5.2

At these dosages all the test substances, with the exception of triadimefon, caused abnormal physical and/or behavioral signs in the rats as monitored in clinical observations. Significant reductions [ $F(1,14) > 7.7$ ,  $p \leq .02$ ] in the number of movements and their pattern within the session occurred in the acrylamide and carbaryl dosage groups. Triadimefon caused a significant increase [ $F(1,14) = 504.7$ ,  $p \leq .001$ ] in the number of movements. The values for the groups administered IDPN and DDT were not significantly different from the respective control group values.



APPENDIX 9  
MOTOR ACTIVITY AND AUDITORY STARTLE  
VALIDATION

**Automated Monitoring Systems for Motor Activity  
and Auditory Startle Applicable for Both  
Developmental and Adult Neurotoxicity Studies**

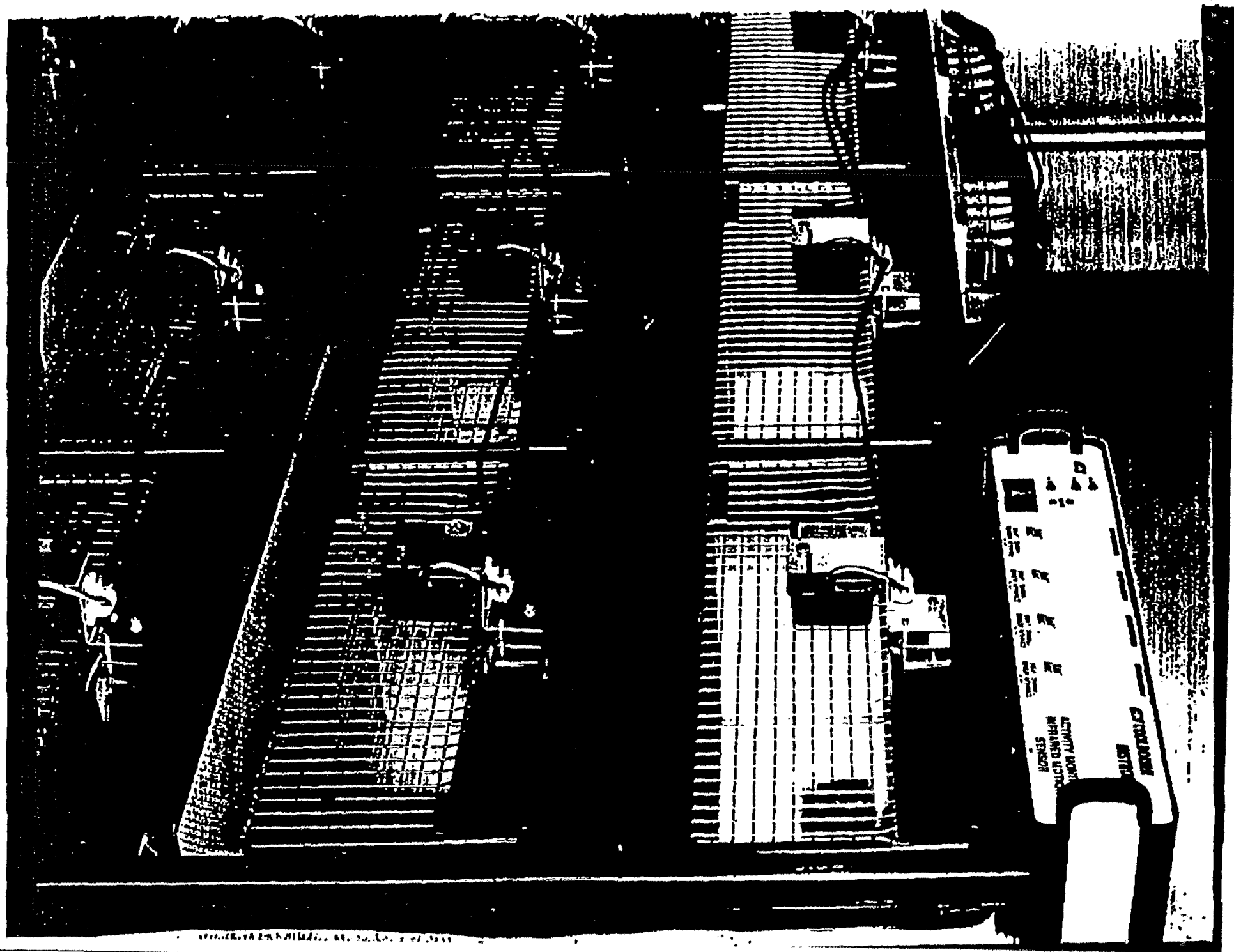
**J. A. Foss, E. A. Lochry and A. M. Hoberman**  
**Argus Research Laboratories, Inc.**

**905 Sheehy Drive  
Horsham, PA 19044  
215-443-8710**

**Poster Presented at the  
8th International Neurotoxicology Conference  
Little Rock, Arkansas  
October, 1990**

## **ABSTRACT**

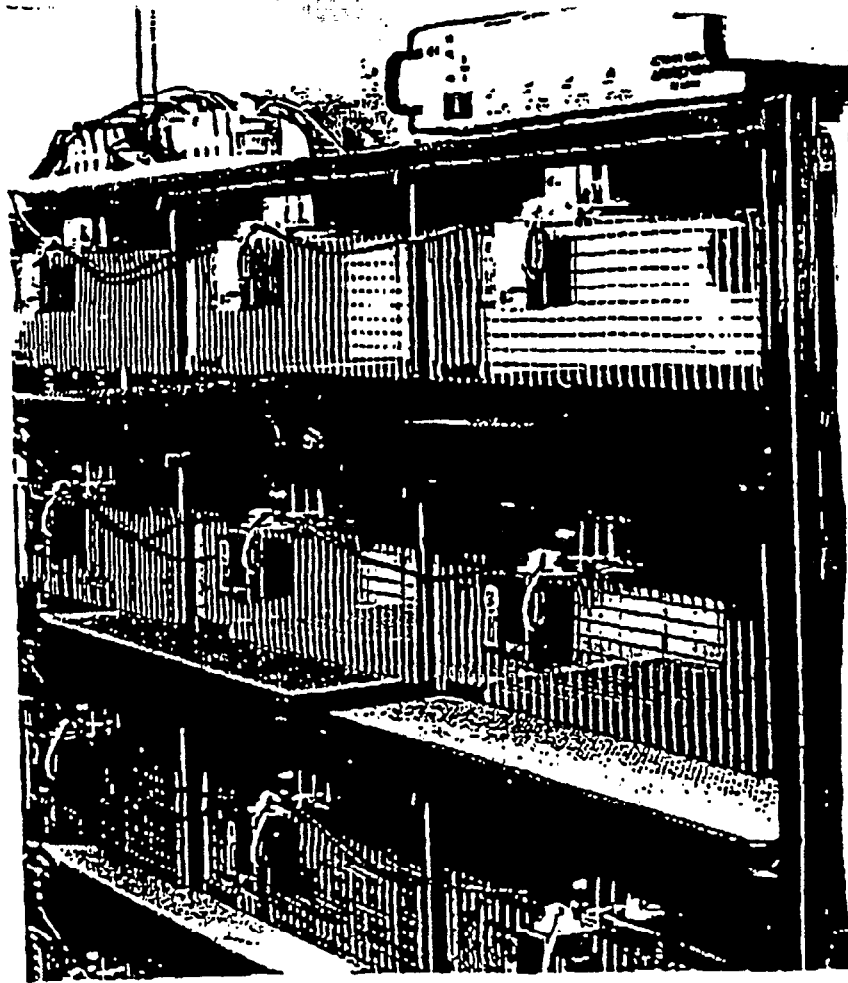
New EPA developmental and adult neurotoxicity guidelines will require testing of large numbers of rodents at specific ages during their growth period. Over the past two years, we have developed and validated, automated, computer-controlled monitoring systems for the collection and compilation of motor activity and auditory startle habituation data for use in both adult and developmental neurotoxicity screens. Motor activity is measured with passive infrared motion detectors in a test apparatus that allows simultaneous monitoring of up to 32 individual rats. Motor activity data will be presented from 16 male and 16 female Crl:CD(TM) VAF/Plus(TM) rats tested in individual stainless-steel cages for 90-minute test periods on postnatal days 13, 17, 21 and 60. A single sensor was attached to each cage, and the continuous output of the sensor was sampled five times each second by a computer-controlled analog-to-digital converter. The number of movements and the total time (seconds) spent in movement were tabulated. The auditory startle response is measured using a force platform, with computer-controlled presentation of auditory startle stimuli and the digital conversion and collection of data. Auditory startle data will be presented from 16 male and 16 female rats tested on postnatal days 22 and 60. During each test session, responses from 50 eliciting stimuli separated by 10-second intervals were recorded. The data collected in both paradigms were analyzed using SAS(TM) and subsequently converted via software programs into GLP-formatted summary and individual tables. The data from both paradigms indicated that these automated, computer-controlled systems are exceptionally well-suited for collecting large quantities of behavioral data on numerous animals at the specific ages required for regulatory studies.



ACTIVITY MONITORING  
WIRELESS LOCATION  
SENSORS

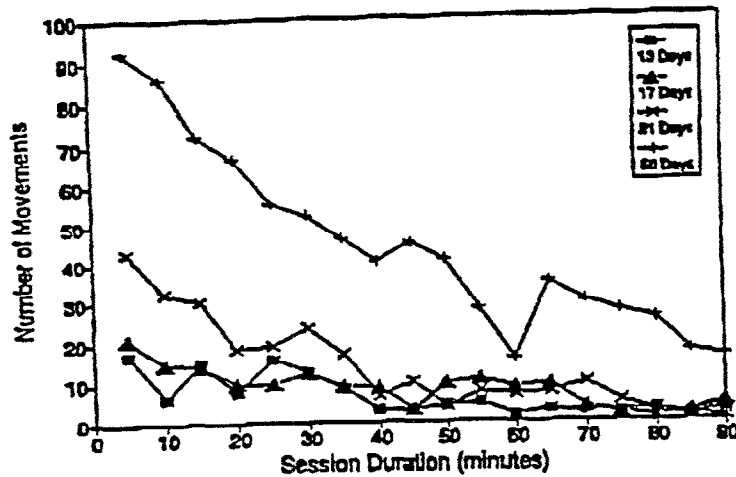
CHINA  
WIRELESS



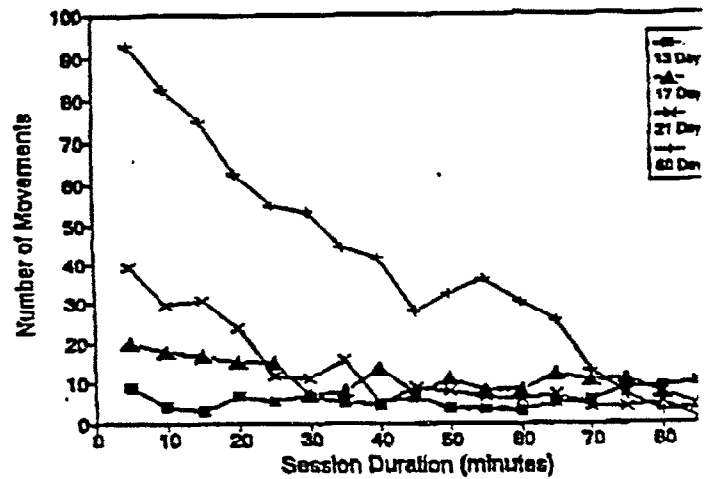


Standard rat cages (double width) were used for the motor activity tests. Stainless steel inserts were placed on the floors when rats were tested at 13, 17 and 21 days of age. The response of the sensor was proportional to the extent of movement and was based on changes in the pattern of heat in the cage. Sets of eight sensors were connected to a distribution box from which a single cable led to the unit containing the analog-to-digital (A/D) converters and power supply; the unit could monitor 32 sensors. The A/D converters sampled the output of the sensors every 200 milliseconds under the control of a microcomputer. Using predefined thresholds, the computer classified the samples as representing no movement, a small movement, or a large movement. Two or more consecutive samples in the same movement category were defined as discrete movements and summed over five minute blocks along with the total time spent in each category. A calibration device and associated software were used to standardize the output of the sensors prior to testing.

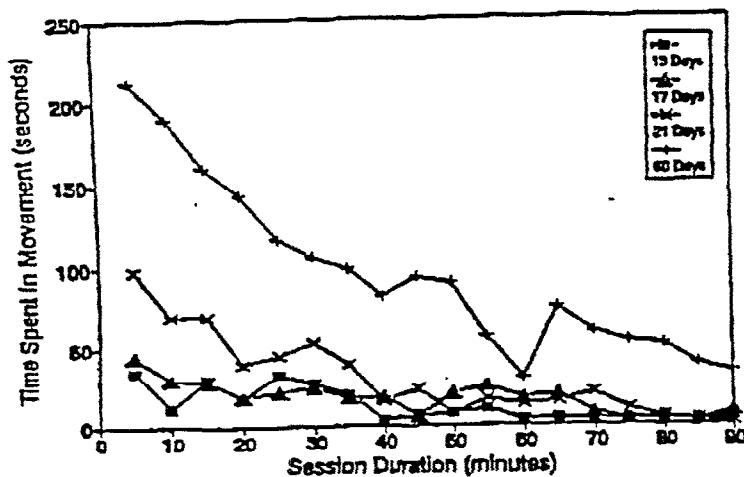
Motor Activity - Females  
Number of Movements



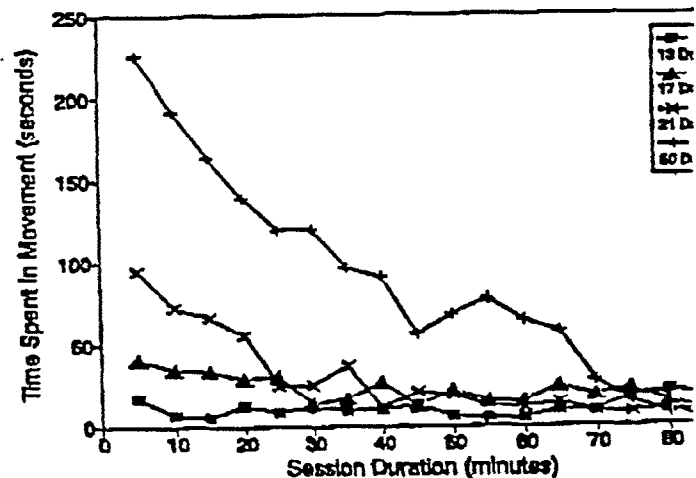
Motor Activity - Males  
Number of Movements



Motor Activity - Females  
Time Spent in Movement



Motor Activity - Males  
Time Spent in Movement



Data for the small and large movement categories were combined for each five minute block. Summaries for the number of movements and time spent in movement showed similar patterns. Both measures decreased significantly over time for the female rats on days 13 through 60 and days 17 through 60 for the males. The smallest coefficients of variation were obtained at 60 days of age: ranging from 30% to 35% for the aggregate scores.

# Motor Activity -- Number of Movements

		Females			Males		
		Mean	S.D.	C.V.	Mean	S.D.	C.V.
Day 13	Total	111.8	85.7	76.7	106.6	100.6	94.4
	5 Minutes	16.9	15.3	90.5	9.0	9.2	102.2
	10 Minutes	6.2	9.3	150.4	4.1	6.3	155.3
	15 Minutes	14.6	18.7	127.5	3.0	4.4	147.1
	20 Minutes	7.4	11.4	153.8	6.8	7.3	108.3
	25 Minutes	15.7	19.6	124.8	5.3	7.5	141.5
	30 Minutes	12.4	17.6	141.9	6.7	9.1	135.5
	35 Minutes	8.3	15.3	184.0	5.2	12.1	233.3
	40 Minutes	2.8	2.6	93.8	4.7	10.3	220.0
	45 Minutes	2.9	4.1	143.6	6.6	18.0	271.2
	50 Minutes	3.8	6.9	181.0	3.8	5.5	146.2
	55 Minutes	4.7	12.8	272.4	3.6	5.0	141.3
	60 Minutes	1.5	1.4	94.3	3.1	4.2	136.2
	65 Minutes	2.7	3.2	119.0	4.9	8.9	181.1
	70 Minutes	2.3	2.9	127.3	5.3	9.4	17.9
	75 Minutes	2.3	3.6	157.8	8.7	14.5	166.6
	80 Minutes	3.0	6.1	203.3	8.7	16.8	193.8
	85 Minutes	1.3	1.5	117.1	9.6	14.9	155.0
	90 Minutes	3.0	4.2	138.8	7.6	16.2	213.1
Day 17	Total	150.8	169.2	112.3	198.5	265.4	133.7
	5 Minutes	20.9	20.1	96.3	20.0	21.7	108.5
	10 Minutes	14.6	15.8	108.2	17.7	18.2	103.2
	15 Minutes	14.1	20.0	141.8	16.6	24.5	147.2
	20 Minutes	9.5	17.2	181.2	15.4	22.5	146.5
	25 Minutes	9.5	21.4	224.7	15.1	21.8	144.4
	30 Minutes	12.1	14.5	120.3	6.8	11.2	166.2
	35 Minutes	8.6	15.2	175.9	7.9	15.2	191.3
	40 Minutes	8.3	15.6	187.3	13.5	25.2	186.8
	45 Minutes	3.1	5.4	177.0	6.9	10.7	154.3

# Motor Activity -- Number of Movements

		Females			Males		
		Mean	S.D.	C.V.	Mean	S.D.	C.V.
Day 21	50 Minutes	9.4	17.2	183.5	10.9	22.6	206.4
	55 Minutes	10.3	20.6	199.4	7.9	12.3	155.5
	60 Minutes	8.3	16.1	194.9	7.8	10.1	129.5
	65 Minutes	9.1	18.1	199.8	12.1	24.0	197.6
	70 Minutes	3.4	8.3	247.2	10.4	21.1	202.9
	75 Minutes	2.0	2.7	132.9	10.9	20.2	186.1
	80 Minutes	1.1	1.9	171.4	7.3	21.9	301.8
	85 Minutes	2.1	3.0	144.3	4.3	9.6	225.3
	90 Minutes	4.6	7.9	173.9	7.0	21.9	313.4
	Total	242.4	170.1	70.2	217.5	149.2	68.6
	5 Minutes	42.6	18.2	42.8	39.3	14.8	37.8
	10 Minutes	32.1	20.6	64.1	29.3	15.8	54.0
	15 Minutes	30.1	20.9	69.2	30.4	16.3	53.6
	20 Minutes	18.4	15.8	86.2	23.6	19.4	82.2
	25 Minutes	19.1	20.9	109.2	11.7	12.8	109.5
	30 Minutes	23.4	24.6	105.1	10.8	15.1	139.4
	35 Minutes	16.9	20.4	120.7	15.8	18.8	119.7
	40 Minutes	6.3	10.1	161.5	4.5	9.9	219.5
	45 Minutes	9.8	14.7	150.5	8.8	14.9	170.4
	50 Minutes	3.6	7.4	208.0	7.9	14.4	181.1
Day 60	55 Minutes	7.4	21.1	286.4	6.3	15.3	244.6
	60 Minutes	6.4	16.7	259.8	5.8	9.3	162.6
	65 Minutes	7.2	14.7	204.4	6.8	13.9	204.2
	70 Minutes	9.4	17.4	185.6	4.0	10.2	255.0
	75 Minutes	5.0	9.3	186.5	3.8	7.4	192.8
	80 Minutes	2.5	4.3	172.2	5.0	13.2	263.7
	85 Minutes	1.6	3.2	197.0	1.1	2.0	180.4
	90 Minutes	0.8	1.4	185.4	2.7	5.1	190.6
	Total	790.4	243.7	30.8	690.8	234.6	34.0

Motor Activity -- Number of Movements						
	Females			Males		
	Mean	S.D.	C.V.	Mean	S.D.	C.V.
5 Minutes	92.1	6.6	7.2	92.8	13.0	14.0
10 Minutes	85.8	11.9	13.8	82.4	11.8	14.4
15 Minutes	72.1	10.1	14.1	74.7	16.9	22.6
20 Minutes	66.6	20.3	30.5	62.5	22.0	35.3
25 Minutes	55.3	28.2	50.9	54.8	23.7	43.3
30 Minutes	52.1	25.7	49.3	52.9	24.2	45.7
35 Minutes	46.3	26.1	56.4	44.3	30.1	67.9
40 Minutes	40.2	31.1	77.3	41.4	28.4	68.6
45 Minutes	45.1	28.9	64.2	27.6	22.9	83.2
50 Minutes	40.6	30.2	74.4	32.1	30.1	93.8
55 Minutes	28.0	24.1	86.1	35.8	27.3	76.3
60 Minutes	15.6	18.1	116.5	29.9	30.5	102.2
65 Minutes	34.8	29.8	85.9	25.1	27.5	109.4
70 Minutes	29.7	26.2	88.3	12.8	18.0	140.4
75 Minutes	27.3	32.7	119.8	7.0	10.8	154.3
80 Minutes	25.4	33.0	129.7	2.9	9.1	311.1
85 Minutes	17.4	24.5	141.0	3.9	10.8	279.4
90 Minutes	16.1	21.5	133.4	8.1	21.5	266.2

The data were analyzed for each age and sex as a repeated measures design using the SAS(TM) GLM procedure. Probability values were adjusted by the Greenhouse-Geisser procedure. The differences in the number of movements throughout the intervals of the session were statistically significant ( $P < .05$ ) on days 13 through 60 for the females and on days 21 and 60 for the males.

Motor Activity -- Time Spent in Movement (sec)							
		Females			Males		
		Mean	S.D.	C.V.	Mean	S.D.	C.V.
Day 13	Total	222.6	200.3	90.0	198.3	211.9	106.9
	5 Minutes	34.7	36.4	104.8	16.9	20.2	119.3
	10 Minutes	11.7	18.2	155.9	7.0	11.6	165.1
	15 Minutes	29.5	46.0	155.9	5.1	7.9	156.4
	20 Minutes	17.8	30.2	170.0	12.4	15.5	124.8
	25 Minutes	32.4	43.3	133.6	8.8	13.8	157.4
	30 Minutes	26.4	40.3	152.7	11.2	17.7	158.0
	35 Minutes	19.2	43.8	228.4	9.9	25.4	255.2
	40 Minutes	3.6	4.1	113.9	9.8	27.0	275.4
	45 Minutes	5.9	11.6	195.0	12.7	37.7	297.5
	50 Minutes	7.1	14.8	209.0	5.9	9.1	155.2
	55 Minutes	10.1	31.4	312.0	4.8	7.1	147.4
	60 Minutes	3.2	3.7	114.7	4.8	7.0	145.6
	65 Minutes	3.6	5.0	136.6	9.1	18.5	204.2
	70 Minutes	3.8	5.0	132.6	8.8	17.3	196.2
	75 Minutes	3.3	5.5	168.0	17.9	35.1	195.9
	80 Minutes	4.6	9.5	207.5	20.8	42.2	202.7
	85 Minutes	1.6	2.0	130.1	18.6	33.5	180.0
	90 Minutes	4.4	7.0	157.4	13.7	30.8	225.3
Day 17	Total	308.1	366.7	119.0	394.4	584.6	148.2
	5 Minutes	44.3	42.3	95.7	41.1	45.9	111.7
	10 Minutes	29.9	33.0	110.4	34.8	43.2	124.1
	15 Minutes	28.4	42.3	148.6	34.4	54.8	159.3
	20 Minutes	18.5	36.4	196.5	29.5	48.6	164.6
	25 Minutes	21.1	48.8	231.0	30.6	48.8	159.7
	30 Minutes	23.6	31.2	131.9	13.1	24.6	187.3
	35 Minutes	17.4	37.4	214.5	16.9	32.1	189.6
	40 Minutes	17.7	38.9	220.0	26.8	49.7	185.4
	45 Minutes	5.8	10.5	183.3	12.2	21.5	176.7

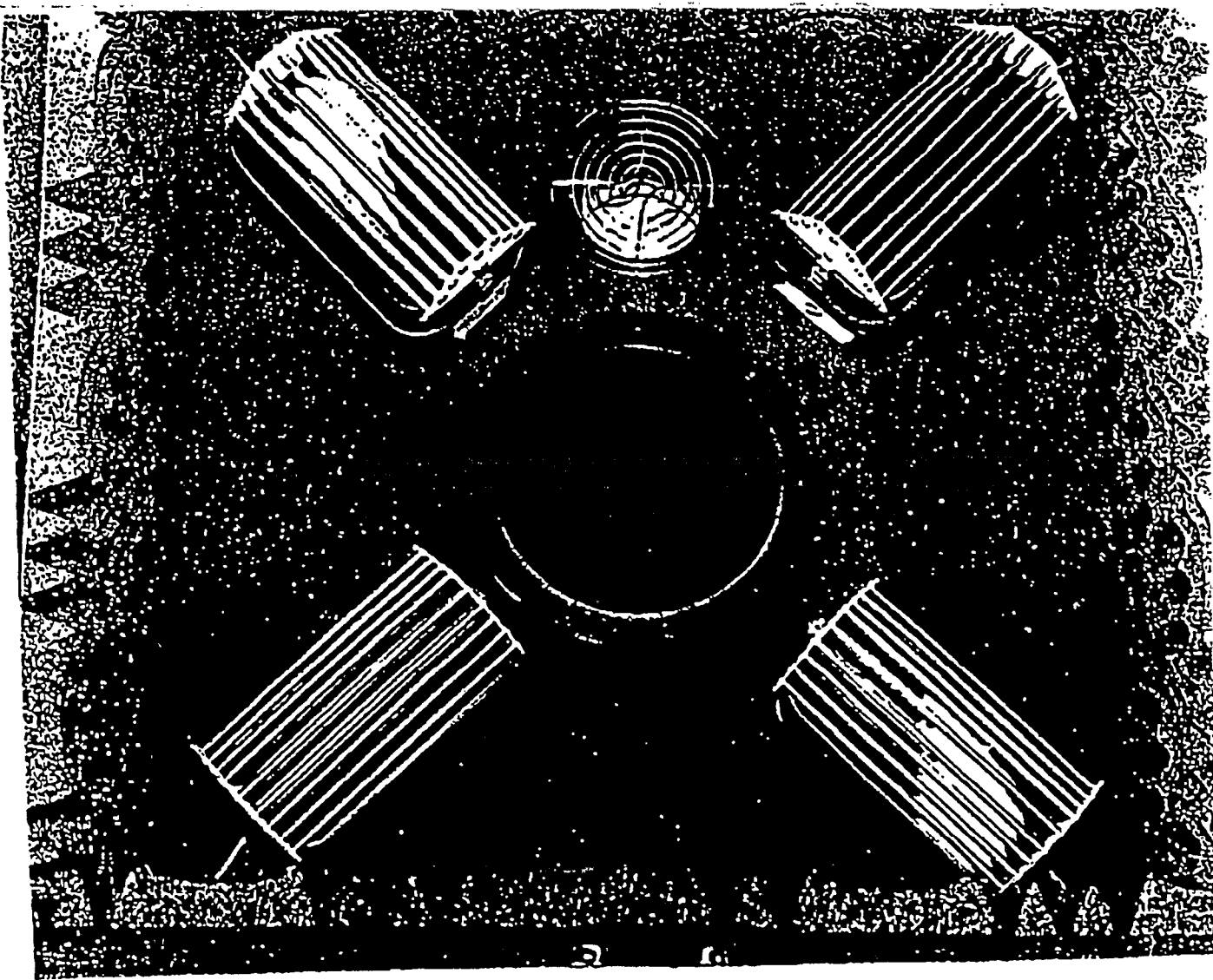
**Motor Activity -- Time Spent in Movement (sec)**

		Females			Males		
		Mean	S.D.	C.V.	Mean	S.D.	C.V.
Day 21	50 Minutes	19.4	37.4	193.2	21.8	49.6	228.0
	55 Minutes	23.6	46.9	199.0	15.1	25.6	170.1
	60 Minutes	16.8	35.0	208.0	15.3	21.8	142.1
	65 Minutes	18.6	39.8	214.6	24.4	53.2	217.6
	70 Minutes	7.1	18.5	260.3	18.8	40.6	216.8
	75 Minutes	3.9	6.2	157.5	23.0	45.5	197.6
	80 Minutes	2.1	3.3	157.4	14.5	49.0	337.9
	85 Minutes	2.5	4.1	164.6	8.3	23.3	280.2
	90 Minutes	7.4	13.7	186.2	13.8	47.3	342.6
	Total	536.4	408.3	76.1	501.0	348.7	69.6
	5 Minutes	98.3	47.5	48.4	94.9	35.4	37.3
	10 Minutes	70.4	50.1	71.1	72.6	49.1	67.5
	15 Minutes	70.2	50.9	72.5	67.3	39.3	58.4
	20 Minutes	39.1	38.6	98.8	56.3	46.9	83.4
	25 Minutes	44.1	53.9	122.4	25.4	30.0	118.1
	30 Minutes	52.4	60.0	114.3	25.4	37.4	147.3
	35 Minutes	38.2	50.5	132.2	37.5	44.2	117.8
	40 Minutes	14.6	24.4	167.1	10.6	26.0	245.9
	45 Minutes	22.1	35.4	159.9	21.3	35.1	165.2
	50 Minutes	7.4	17.5	235.5	18.8	33.6	178.7
	55 Minutes	15.9	44.6	279.8	12.6	35.1	278.0
	60 Minutes	12.6	33.2	263.9	10.9	17.8	163.8
	65 Minutes	14.3	29.2	204.3	14.1	29.2	206.9
	70 Minutes	19.1	35.4	185.7	8.3	21.2	255.2
	75 Minutes	9.8	18.5	188.0	7.8	18.9	243.5
	80 Minutes	3.9	6.8	176.3	9.8	30.1	308.3
	85 Minutes	2.9	6.7	226.7	2.1	3.8	181.8
	90 Minutes	1.1	2.2	194.4	5.4	11.7	217.2
Day 60	Total	1676.9	496.7	29.6	1551.8	550.7	35.5

Motor Activity -- Time Spent in Movement (sec)						
	Females			Males		
	Mean	S.D.	C.V.	Mean	S.D.	C.V.
5 Minutes	212.6	23.5	11.1	225.6	25.8	11.4
10 Minutes	190.0	20.8	10.9	191.2	29.1	15.2
15 Minutes	160.9	31.6	19.6	163.4	37.1	22.7
20 Minutes	143.6	44.5	31.0	138.9	49.7	35.8
25 Minutes	116.6	66.6	57.1	120.0	58.9	49.1
30 Minutes	105.6	51.4	48.7	119.7	58.4	48.8
35 Minutes	98.4	60.6	61.5	96.8	68.7	71.0
40 Minutes	81.2	65.5	80.6	91.2	63.8	70.0
45 Minutes	91.6	59.8	65.2	57.3	48.9	85.4
50 Minutes	88.1	67.3	76.3	68.9	60.7	88.1
55 Minutes	55.5	48.5	87.5	77.9	61.6	79.0
60 Minutes	29.4	34.7	118.0	65.5	72.9	111.3
65 Minutes	73.4	71.9	98.0	58.1	65.5	112.7
70 Minutes	58.5	50.5	86.4	28.0	42.7	152.7
75 Minutes	52.3	65.2	124.7	15.0	23.3	155.0
80 Minutes	49.7	67.0	134.8	6.7	19.6	293.3
85 Minutes	37.4	55.3	147.6	9.1	25.9	285.6
90 Minutes	32.1	43.3	134.8	18.6	49.9	268.8

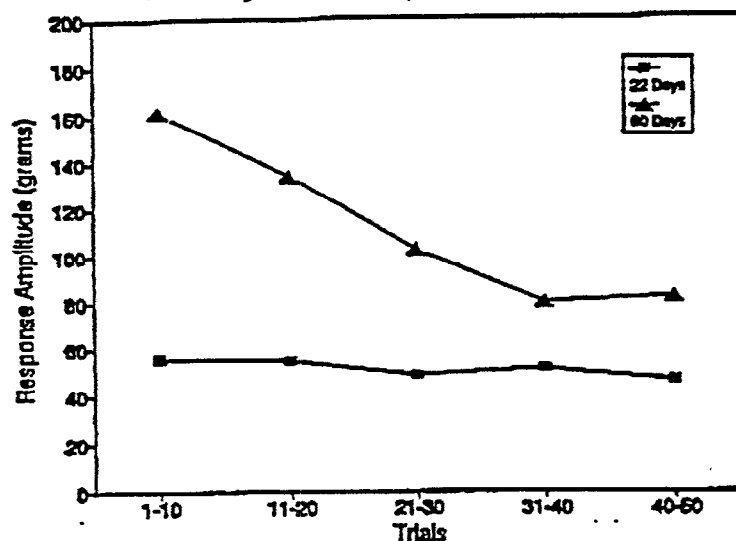
The data were analyzed for each age and sex as a repeated measures design using the SAS(TM) GLM procedure. Probability values were adjusted by the Greenhouse-Geisser procedure. The differences in the time spent in movement throughout the intervals of the session were statistically significant ( $P < .05$ ) on days 13 through 60 for the females and on days 21 and 60 for the males.



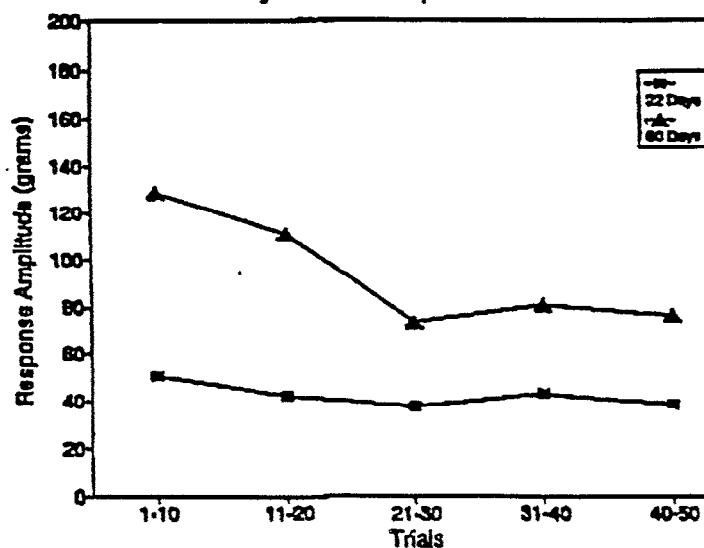


Rats were placed in holders and then set on top of force platforms for the auditory startle test. Prior to testing, standard weights were used to compare the output of the platforms, and adjustments made in the amplification of each sensor to calibrate them. The four platforms were enclosed in a sound-attenuating chamber with a constant 70 dB background of low-frequency noise. The fifty eliciting stimuli were 20 millisecond bursts of noise with a peak intensity of 120 dB and were separated by 10 second intervals. The output of each platform was sampled by an analog-to digital converter at 1 millisecond intervals for 200 milliseconds following the stimulus, and the peak value used as the basic measure of the startle response.

**Auditory Startle Response - Females**



**Auditory Startle Response - Males**

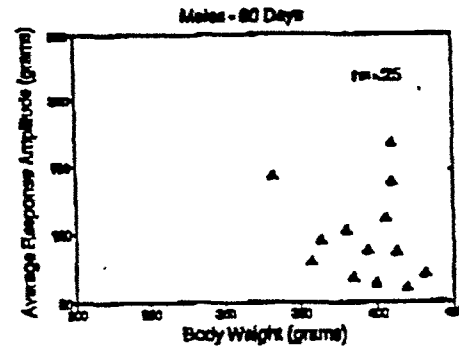
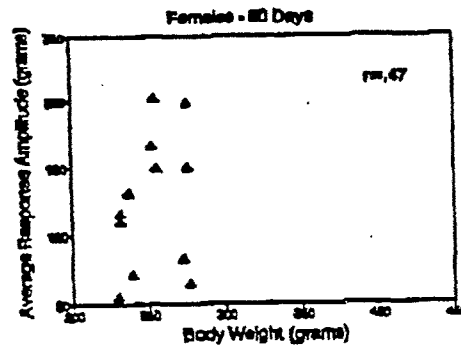
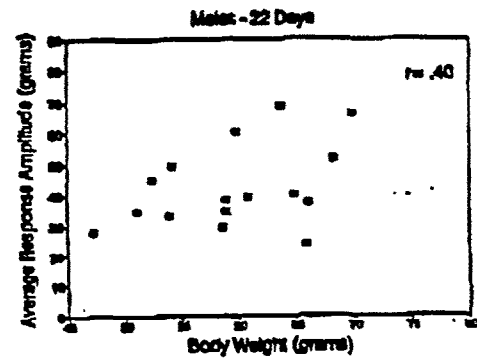
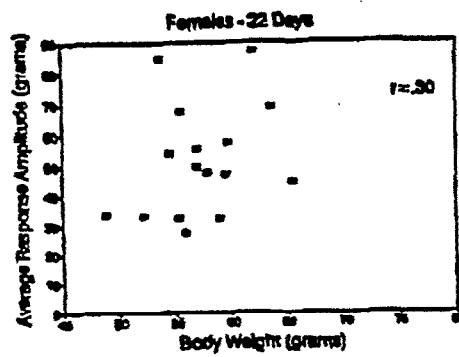


The peak responses were averaged over ten trial intervals and converted to grams. The rat's body weight was then subtracted from each score to obtain a measure of response amplitude. Neither the males nor females showed evidence of habituation, a decrease in response amplitude over trials, at 22 days; however, there was a strong trend for both sexes at 60 days. The smallest coefficients of variation were obtained at 22 days: 36% for the females and 32% for the males based on the aggregate scores.

Auditory Startle Habituation							
Females				Males			
		Mean	S.D.	C.V.	Mean	S.D.	C.V.
Day 22	Body Weight	57.4	4.2	7.4	59.8	6.6	11.0
	Average Response	51.4	18.3	35.7	42.4	13.4	31.6
	Trials 1-10	55.6	24.4	43.9	51.0	14.2	27.9
	Trials 11-20	54.9	21.3	38.8	42.1	13.3	31.7
	Trials 21-30	48.6	20.5	42.2	37.6	12.9	34.3
	Trials 31-40	51.4	23.9	46.5	42.8	17.7	41.3
	Trials 41-50	46.5	20.5	44.0	38.8	23.5	60.6
Day 60	Body Weight	249.6	19.7	7.9	393.6	27.5	7.0
	Average Response	112.1	57.0	50.9	93.7	36.5	38.9
	Trials 1-10	161.5	105.6	65.4	128.9	65.7	51.0
	Trials 11-20	134.4	78.6	58.5	110.3	60.1	54.5
	Trials 21-30	102.5	55.7	54.4	73.1	31.2	42.6
	Trials 31-40	79.8	39.5	49.6	80.1	29.6	36.9
	Trials 41-50	82.2	35.2	42.8	76.1	32.5	42.7

The data were analyzed for each age and sex as a repeated measures design using the SAS(TM) GLM procedure. Probability values were adjusted by the Greenhouse-Geisser procedure. The differences in response amplitude throughout the blocks of trials were statistically significant ( $P < .05$ ) on day 22 (the linear contrast was not significant) and day 60 for the males and on day 60 for the females.

## Body Weight Versus Auditory Startle Response



Responses were not significantly correlated with body weight for either sex at any age.

**APPENDIX 10**  
**DEVELOPMENTAL NEUROTOXICITY**  
**EVALUATION OF LEAD NITRATE**

**(MOTOR ACTIVITY, PASSIVE AVOIDANCE, AUDITORY STARTLE  
AND WATERMAZE)**

# **Developmental Neurotoxicity Evaluation of Lead Nitrate in Crl:CD<sup>®</sup>BR VAF/Plus<sup>®</sup> Rats**

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**215-443-8710**

**Poster Presented at the**

**Annual Meeting of the Society of Toxicology**

**Seattle, Washington**

**February, 1992**

## ABSTRACT

The EPA guidelines for a developmental neurotoxicity study were used as the basis for an evaluation of lead nitrate. Pregnant rats were given the test substance orally by gavage from day 6 of gestation through day 10 of lactation at dosages of 0(Vehicle), 5 and 50 mg/kg/day. The high dosage caused significant increases in the incidences of clinical observations in the dams and significantly reduced the feed consumption values throughout gestation and the early lactation period. Significant numbers of pups in the high dosage group died on days 1 to 4 postpartum and average pup weights per litter were reduced in that group on days 1 to 7 postpartum. Significant differences in body weights and feed consumption values also occurred in the high dosage group F1 generation rats during the early postweaning period. There were no differences among the three dosage groups in the functional evaluations of the F1 generation rats: motor activity monitored for one hour periods on days 13, 17, 21 and 60( $\pm$ 2) days postpartum, auditory startle habituation measured on 22 and 60( $\pm$ 2) days postpartum, performance in a passive avoidance task during the early postweaning period, and performance of adult rats in an M-maze watermaze task. Brain weight to body weight ratios measured in day 11 postpartum pups and in adult rats, as well as the indices of sexual maturation, were unaffected by the low and high dosages of the test substance. Neurohistological examination of high dosage group adult rats did not reveal any pathology. [We thank R H Garman (Consultants in Veterinary Pathology) for the microscopic evaluation.]

## Significant Clinical Observations<sup>a</sup>

### Fo Generation Rats

	Gestation Period (mg/kg/day)			Lactation Period (mg/kg/day)		
	0	5	50	0	5	50
	(Vehicle)			(Vehicle)		
Maximum Possible Incidence <sup>b</sup>	554/ 25	549/ 25	550/25	504/ 24	525/ 25	525/ 25
Ptosis	0/ 0	0/ 0	6/ 4**	0/ 0	1/ 1	1/ 1
Labored Breathing	0/ 0	0/ 0	5/ 4**	0/ 0	0/ 0	0/ 0
Excess Salivation	0/ 0	0/ 0	4/ 3**	0/ 0	0/ 0	0/ 0
Decreased Motor Activity	0/ 0	0/ 0	3/ 3**	0/ 0	0/ 0	1/ 1
Localized Alopecia: Underside	0/ 0	0/ 0	13/ 3**	0/ 0	0/ 0	39/ 3
Clonic Convulsion	0/ 0	0/ 0	0/ 0	0/ 0	1/ 1	0/ 0
Emaciation	0/ 0	0/ 0	0/ 0	0/ 0	0/ 0	3/ 3
Vaginal Area: Black Substance	0/ 0	0/ 0	0/ 0	0/ 0	0/ 0	3/ 3

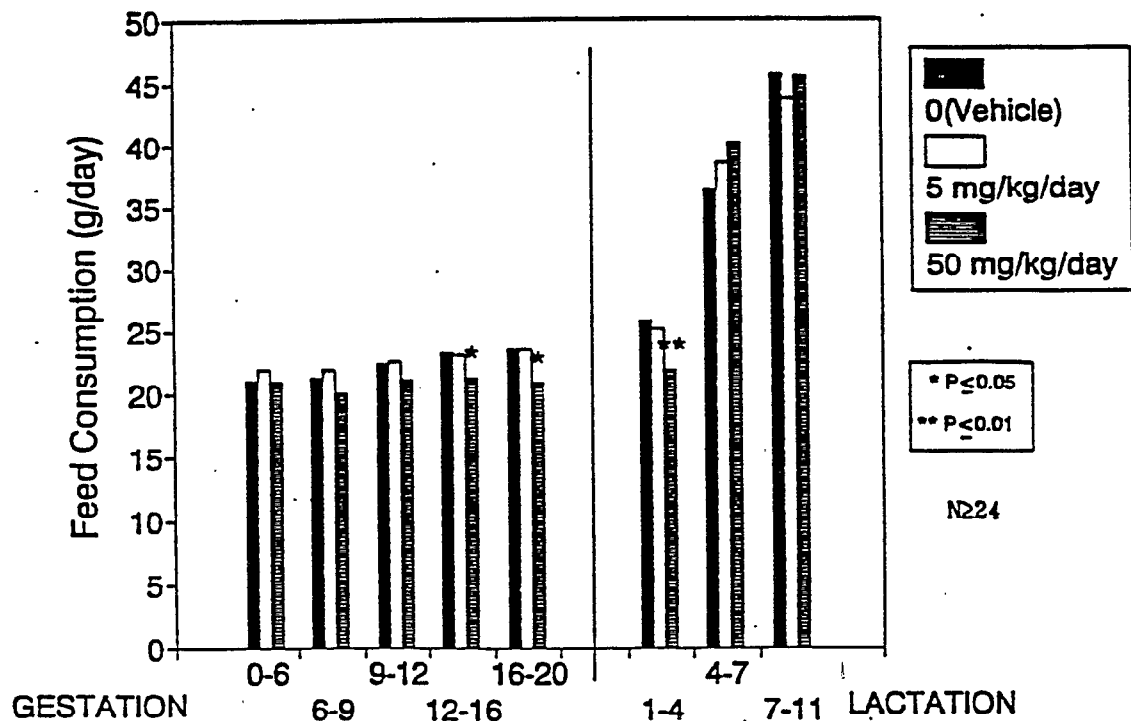
<sup>a</sup> Statistical analyses of clinical observation data were restricted to the number of rats with observations.

<sup>b</sup> Maximum Possible Incidence = (Days x Rats)/Number of Rats Examined per Group

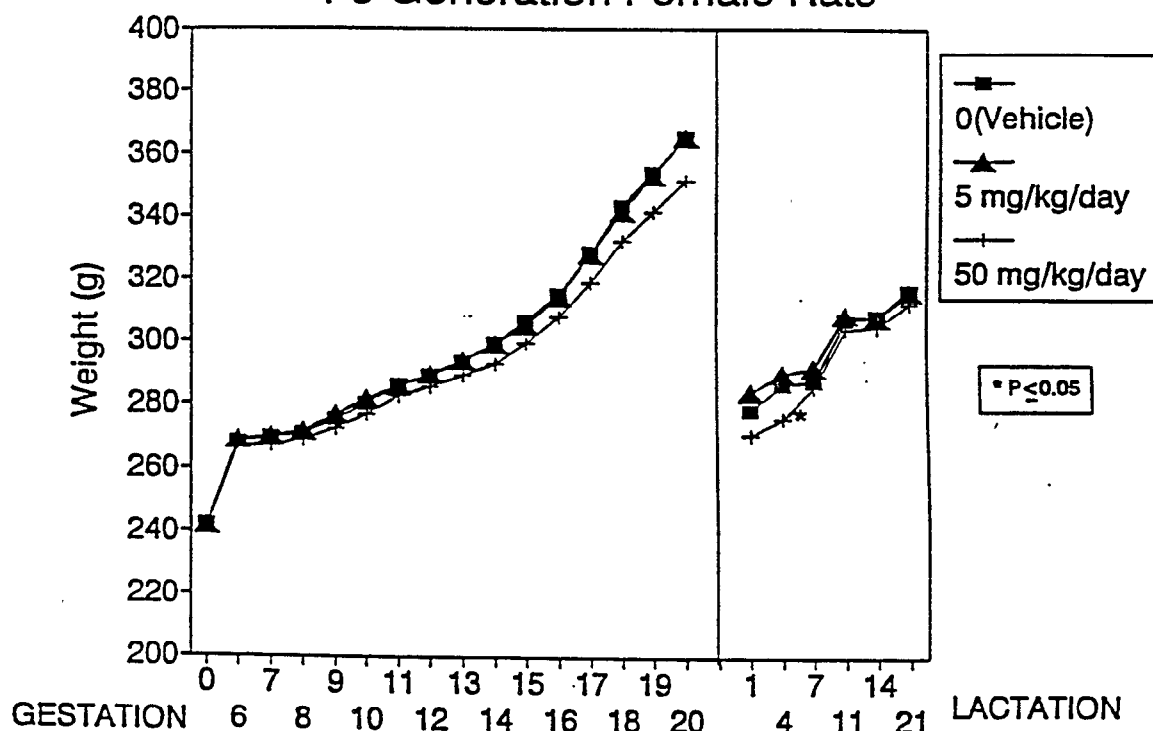
\*\* Significantly different from the vehicle control number ( $P \leq 0.01$ ).



## Maternal Absolute Feed Consumption Fo Generation Female Rats



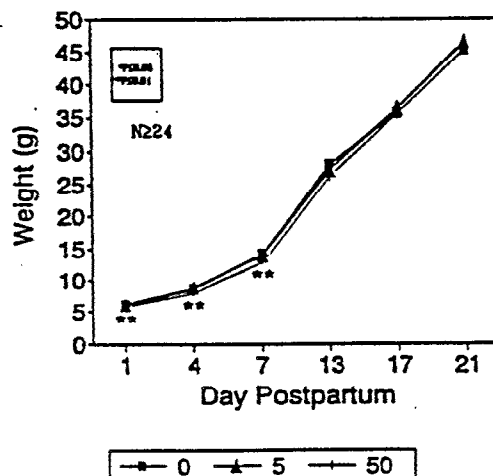
## Maternal Body Weights Fo Generation Female Rats



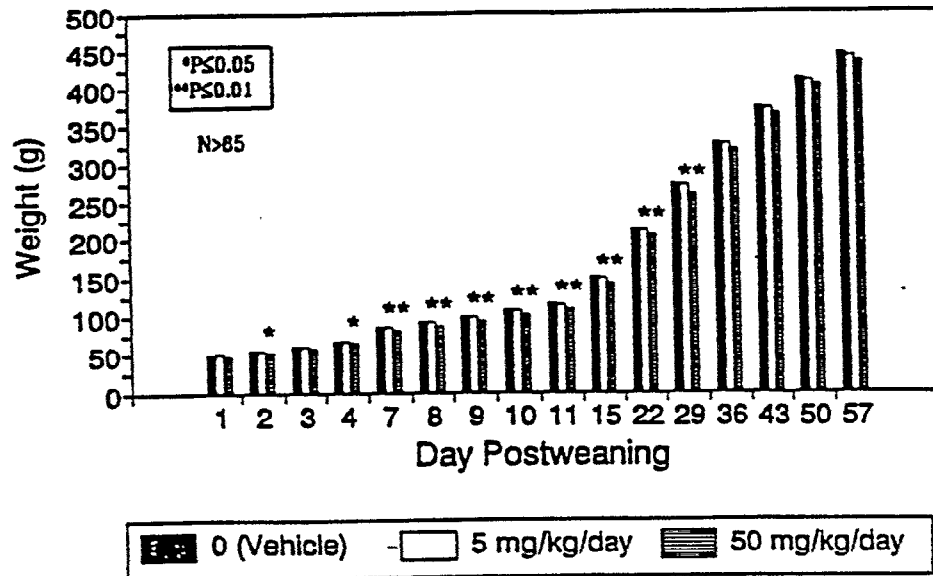
		Dosage (mg/kg/day)		
		0 (Vehicle)	5	50
Rats Pregnant	N	24	25	25
Duration of Gestation in Days	Mean ±S.D.	23.0±0.0	23.0±0.4	23.0±0.3
Pups Delivered	Mean ±S.D.	13.7±2.1	13.1±2.9	13.2±2.4
Liveborn	Mean ±S.D.	13.7±2.1	13.0±2.9	12.9±2.7
Stillborn	Mean ±S.D.	0.04±0.2	0.04±0.2	0.32±1.2
Dams with Stillborn Pups	N(%)	1 (4.2)	1 (4.0)	3 (12.0)
Dams with all Liveborn Pups Dying Days 1 to 4 Postpartum	N(%)	0	0	3 (12.0)
Dams with all Liveborn Pups Dying Days 5 to 21 Postpartum	N(%)	0	0	0
Pups Dying				
Day 1	N(%)	1 (0.3)	1 (0.3)	20 (6.2)**
Days 2-4	N(%)	3 (0.9)	4 (1.2)	21 (6.9)**
Days 5-7	N(%)	0	0	0
Days 8-14	N(%)	0	1 (0.3)	0
Days 15-21	N(%)	0	0	0

\*\* Significantly different from the vehicle control value ( $P \leq 0.01$ ).

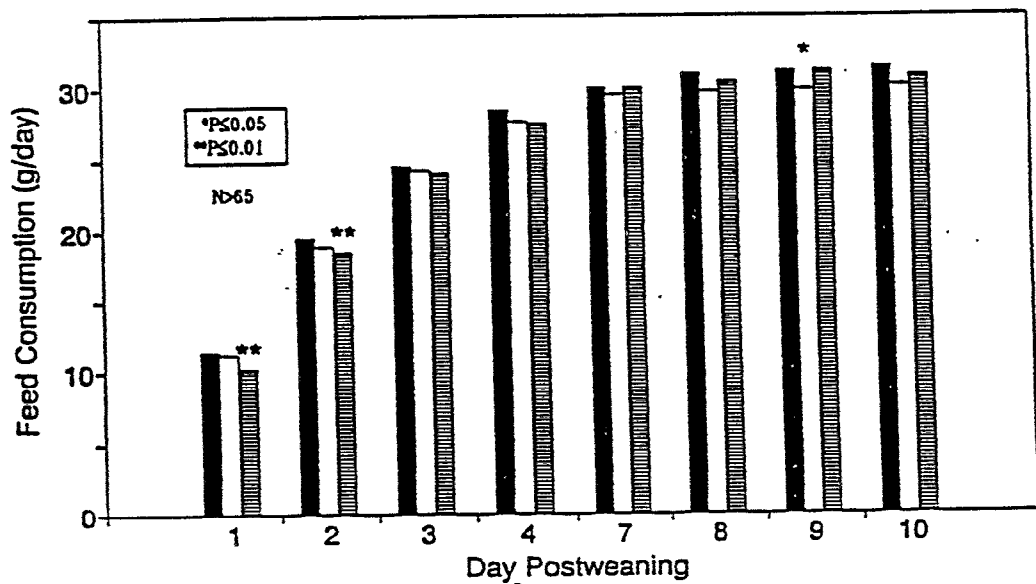
### Pup Weight/Litter F1 Generation Litters



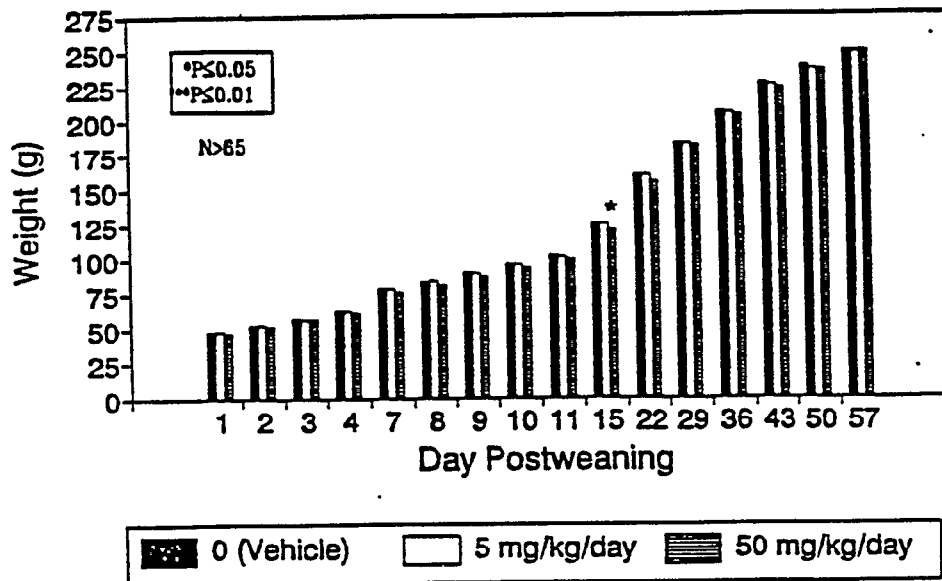
## Postweaning Body Weights F1 Generation Male Rats



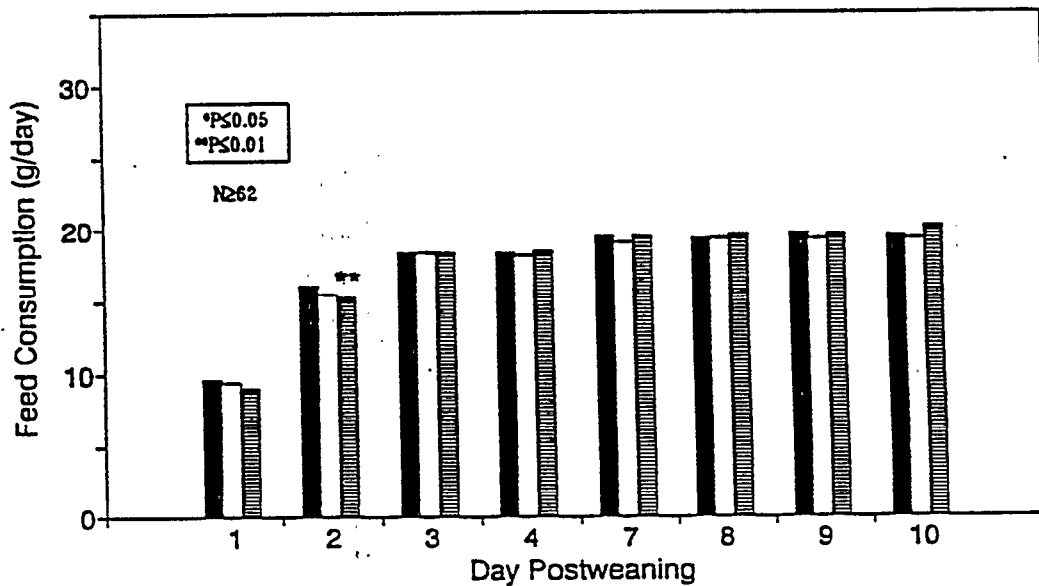
## Postweaning Absolute Feed Consumption F1 Generation Male Rats



## Postweaning Body Weights F1 Generation Female Rats



## Postweaning Absolute Feed Consumption F1 Generation Female Rats

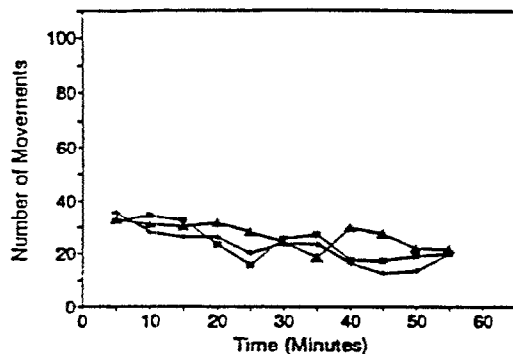


# Motor Activity Evaluation

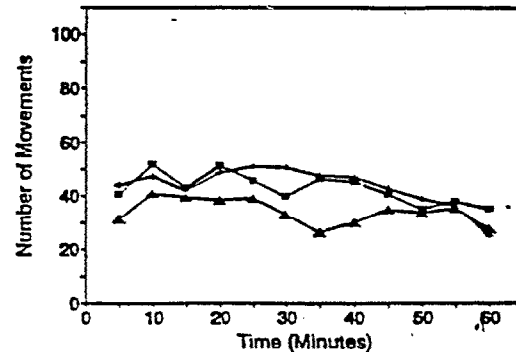
## F1 Generation Rats

One male and one female pup from each litter (N=22 for each dosage group) were individually placed in double-sized laboratory cages for one hour periods on days 13, 17, 21, 58 or 59 postpartum. A metal insert was placed on the grid floor when the rats were 21 days postpartum or less. Activity was monitored by a passive infrared sensor and movements and their durations were compiled in five minute intervals (only the data numbers of movements are shown above). The data were analyzed by repeated measures analyses of variance at each age. The differences between the intervals were statistically significant ( $P \leq 0.001$ ) at each age, but the differences between the dosage groups were not significant.

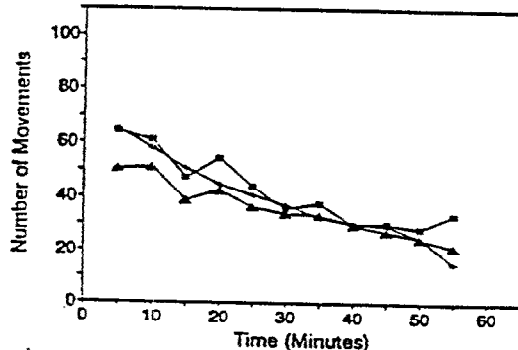
Day 13 Postpartum Male Rats



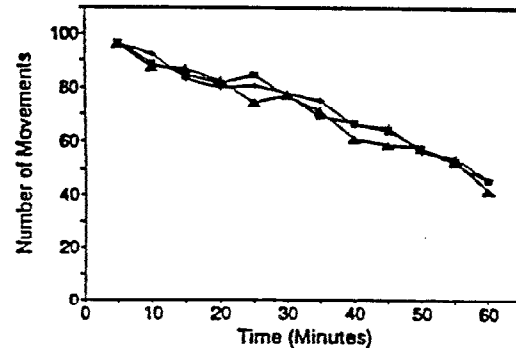
Day 17 Postpartum Male Rats



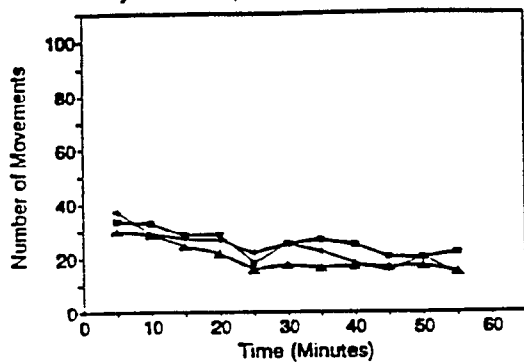
Day 21 Postpartum Male Rats



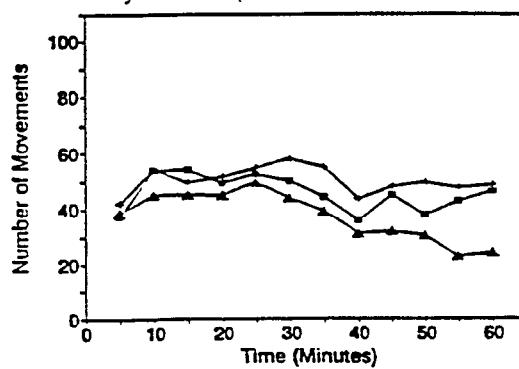
Day 58/59 Postpartum Male Rats



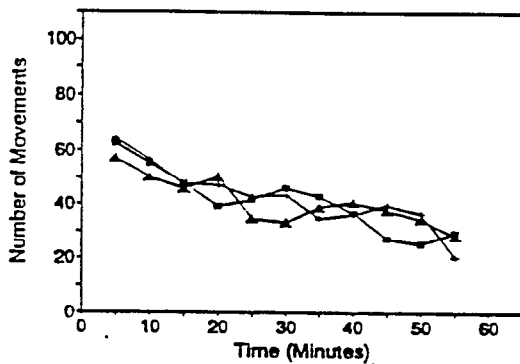
Day 13 Postpartum Female Rats



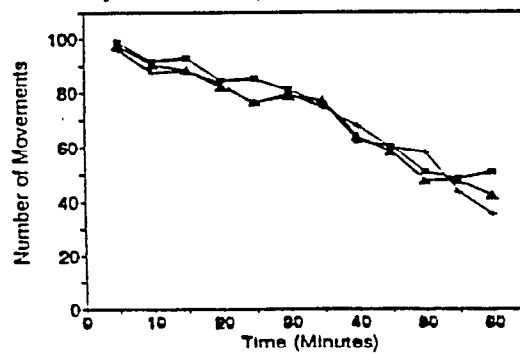
Day 17 Postpartum Female Rats



Day 21 Postpartum Female Rats



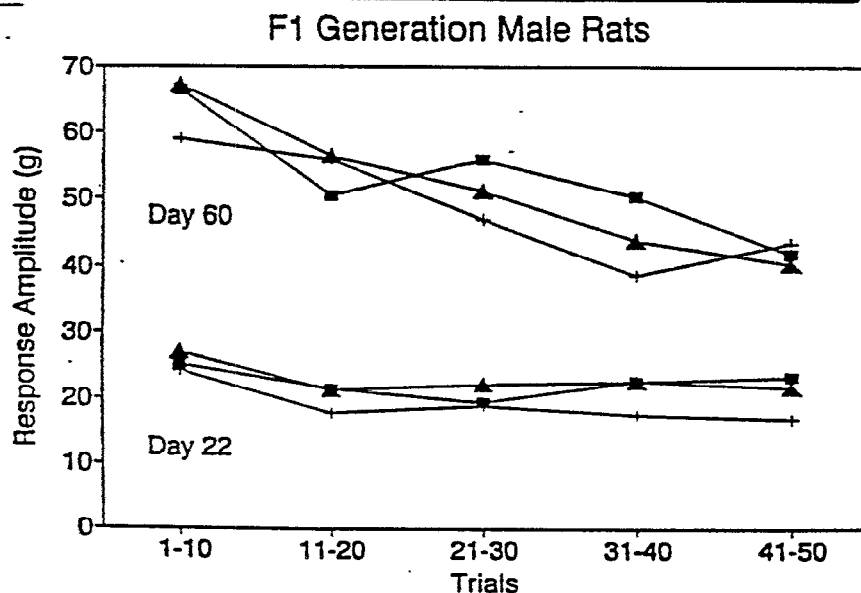
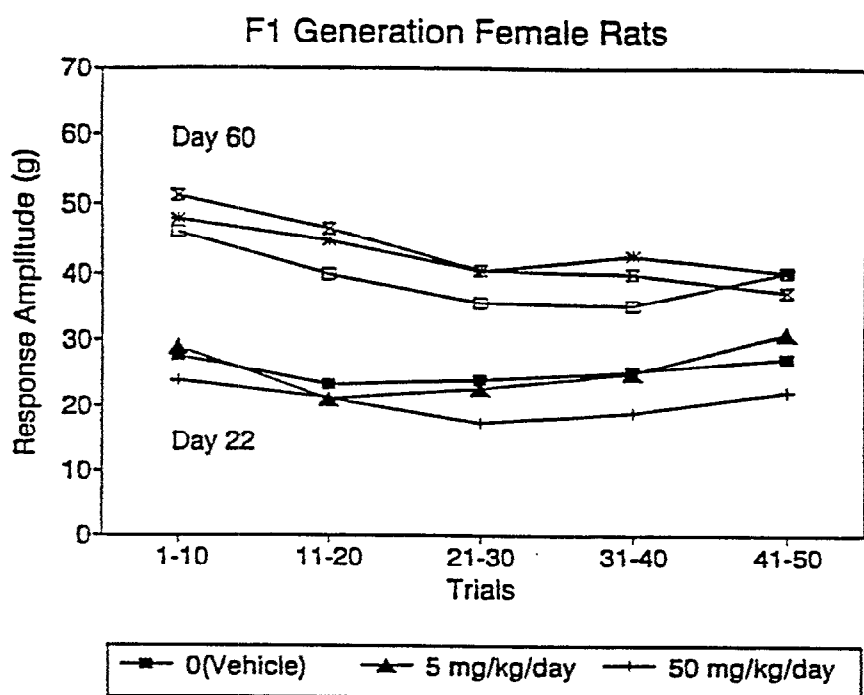
Day 58/59 Postpartum Female Rats



# Auditory Startle Habituation

## F1 Generation Rats

One male and one female rat from each litter (N=22 for each dosage group) were tested on day 22 and day 60 postpartum. The eliciting stimuli were 20 ms, 120 dB bursts of noise and responses were measured by recording the force exerted on a platform in the 200 ms following a stimulus. Fifty stimuli were presented at 10 second intervals and the peak force recorded after each stimulus was subtracted from the average force (body weight) recorded in the absence of a stimulus to obtain the response amplitude. Responses were averaged over ten trial blocks and the data analyzed by repeated measures analyses of variance at each age. The differences between the intervals were statistically significant ( $P \leq 0.001$ ) at each age, but habituation was more pronounced in the adult rats. Differences between the dosage groups were not significant.



# Passive Avoidance Test

## F1 Generation Rats

Beginning on days 23 or 24 postpartum one male and one female rat from each litter were tested in a passive avoidance task. The apparatus consisted of a two-compartment chamber separated by a sliding door. On each trial, a rat was placed into the compartment containing a light. The door was opened and the rat was allowed to explore the apparatus until it entered the "dark" compartment. The sliding door was closed, and a brief pulse of shock was delivered through the floor. The rat was removed for 30 seconds before the start of the next trial. The latency to enter the dark compartment or the maximum 60-second interval was recorded for each trial.

MALE RATS:		Dosage (mg/kg/day)		
		0(Vehicle)	5	50
Session I a:				
Number of Rats	N	22	22	22
Latency Trial 1 b	Mean±S.D.	7.0±4.0	6.0±4.2	6.1±3.9
Latency Trial 2 b	Mean±S.D.	26.4±21.3	21.3±17.4	21.1±18.2
Trials to Criterion	Mean±S.D.	5.0±2.3	4.8±1.1	5.7±3.3
Failed to Learn c	N(%)	0(0.0)	0(0.0)	1(4.5)
Session II a:				
Number of Rats	N	22	22	21
Latency Trial 1 b	Mean±S.D.	26.4±21.3	21.4±17.4	21.1±18.2
Trials to Criterion	Mean±S.D.	3.2±1.8	3.4±2.6	3.2±0.6

- a. Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one-week interval.  
b. The latency was recorded in seconds.  
c. Number of rats that did not meet the criterion on day 1 of testing (learning); day 2 of testing (retention) values for these rats were excluded from group averages and statistical analyses.

FEMALE RATS:		Dosage (mg/kg/day)		
		0(Vehicle)	5	50
Session I a:				
Number of Rats	N	22	22	22
Latency Trial 1 b	Mean±S.D.	5.0±3.9	5.5±4.9	4.8±4.1
Latency Trial 2 b	Mean±S.D.	26.1±22.7	22.0±19.4	17.2±14.0
Trials to Criterion	Mean±S.D.	5.9±3.2	4.8±1.1	5.1±1.2
Failed to Learn c	N(%)	2(9.0)	0(0.0)	0(0.0)
Session II a:				
Number of Rats	N	20	22	22
Latency Trial 1 b	Mean±S.D.	24.5±21.1	27.9±23.4	23.5±22.2
Trials to Criterion	Mean±S.D.	3.6±2.7	3.0±0.7	23.3±1.3

- a. Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one-week interval.  
b. The latency was recorded in seconds.  
c. Number of rats that did not meet the criterion on day 1 of testing (learning); day 2 of testing (retention) values for these rats were excluded from group averages and statistical analyses.



# Watermaze Test

## F1 Generation Rats

Beginning on days 58 or 59 postpartum, one male and one female rat from each litter were evaluated in a water-filled M-maze. On each trial the rat was placed into the base of the M-maze stem farthest from the two arms) and required to swim to one of the two goals c the M-maze in order to be removed from the water. On the first trial, the rat was required to enter both arms of the maze before being removed. The initial arm chosen on Trial 1 was designated the incorrect goal during the remaining trials. Rats that failed to make the correct goal within 60 seconds in any given trial were guided to the correct goal and removed. Each rat was required to reach a criterion of five consecutive errorless trials to terminate the session. The maximum number of trials in any session was 15. Latency (measured in seconds) to choose the correct goal or the maximum 60-second interval was recorded for each trial, as was the number of errors (incorrect turns in the maze) during each trial. Each rat was tested twice, with the sessions separated by a one-week interval. The correct goal and criterion were the same for both sessions.

MALE RATS:		Dosage (mg/kg/day)		
		0(Vehicle)	5	50
Session I a:				
Number of Rats	N	22	22	22
Total Trials	Mean±S.D.	10.6±2.8	9.8±2.4	10.1±3.5
Errors Per Trial	Mean±S.D.	0.5±0.1	0.5±0.2	0.5±0.3
Failed to Learn b	N(%)	2(9.1)	1(4.5)	1(4.5)
Session II a:				
Number of Rats	N	20	21	21
Total Trials	Mean±S.D.	6.5±2.4	7.3±2.7	6.5±1.7
Errors Per Trial	Mean±S.D.	0.1±0.2	0.2±0.2	0.1±0.1

- a. Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one-week interval.  
b. Number of rats that did not meet the criterion on day 1 of testing (learning); day 2 of testing (retention) values for these rats were excluded from group averages and statistical analyses.

FEMALE RATS:		Dosage (mg/kg/day)		
		0(Vehicle)	5	50
Session I a:				
Number of Rats	N	22	22	22
Total Trials	Mean±S.D.	9.2±2.9	9.1±2.0	9.4±2.6
Errors Per Trial	Mean±S.D.	0.4±0.2	0.5±0.2	0.4±0.2
Failed to Learn b	N(%)	1(4.5)	1(4.5)	1(4.5)
Session II a:				
Number of Rats	N	21	21	21
Total Trials	Mean±S.D.	7.4±2.8	7.5±2.7	7.4±3.2
Errors Per Trial	Mean±S.D.	0.2±0.2	0.1±0.2	0.1±0.2

- a. Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one-week interval.  
b. Number of rats that did not meet the criterion on day 1 of testing (learning); day 2 of testing (retention) values for these rats were excluded from group averages and statistical analyses.

## SEXUAL MATURATION

		Dosage (mg/kg/day)		
		0(Vehicle)	5	50
MALE RATS:	N	66	65	64 a
Preputial Separation b	Mean±S.D.	45.2±2.1	45.8±3.4 [63] c	46.1±2.5 [63] c
No Preputial Separation d	N	0	2	1
FEMALE RATS:	N	65	62	65
Vagina Patent b	Mean±S.D.	31.3±1.6	31.4±1.8	31.2±1.6

- a. Excludes values for one rat, which was moribund sacrificed on day 2 postpartum.  
b. Average day postpartum that the prepuce were first observed to be separated or the vagina was patent.  
c. Excludes values for rats with no preputial separation before scheduled sacrifice.  
d. Rats with no preputial separation before scheduled sacrificed (73 days of age).

## BRAIN WEIGHTS - F1 GENERATION PUPS (DAY 11 POSTPARTUM)

		Dosage (mg/kg/day)		
		0(Vehicle)	5	50
Litters evaluated	N	17	16	16
Pups evaluated	N	20	20	20
MALE RATS:	Mean±S.D.	1.06±0.05 [10]	1.05±0.06 [10]	0.99±0.06 [10]
FEMALE RATS:	Mean±S.D.	1.00±0.05 [10]	0.98±0.05 [10]	0.97±0.06** [10]

[ ] = Number of values averaged.

\*\* Significantly different from the vehicle control group value ( $P \leq 0.01$ ).

## BRAIN WEIGHTS AND TERMINAL BODY WEIGHTS - F1 GENERATION RATS (TERMINATION)

		Dosage (mg/kg/day)		
		0(Vehicle)	5	50
MALE RATS:	N	11	11	11
Terminal Body Weight	Mean±S.D.	465.9±28.0	465.3±37.0	453.9±46.5
Whole Brain	Mean±S.D.	2.101±0.110	2.038±0.086	2.039±0.074
Telencephalon	Mean±S.D.	1.244±0.053	1.206±0.059	1.199±0.070
Diencephalon/Mid-Brain	Mean±S.D.	0.284±0.021	0.271±0.026	0.286±0.014
Cerebellum	Mean±S.D.	0.295±0.025	0.281±0.030	0.278±0.013
Medulla Oblongated/Pons	Mean±S.D.	0.253±0.030	0.263±0.019	0.255±0.019
FEMALE RATS:	N	11	11	11
Terminal Body Weight	Mean±S.D.	249.4±26.0	257.3±23.6	257.2±25.2
Whole Brain	Mean±S.D.	1.875±0.049	1.978±0.109**	1.858±0.080
Telencephalon	Mean±S.D.	1.108±0.033	1.178±0.081*	1.093±0.064
Diencephalon/Mid-Brain	Mean±S.D.	0.268±0.019	0.267±0.020	0.259±0.017
Cerebellum	Mean±S.D.	0.257±0.015	0.274±0.018*	0.257±0.021
Medulla Oblongated/Pons	Mean±S.D.	0.232±0.018	0.245±0.022	0.238±0.015

All Weights were recorded in grams (g).

\* Significantly different from the vehicle control group value ( $P \leq 0.05$ ).

\*\* Significantly different from the vehicle control group value ( $P \leq 0.01$ ).

## **ACKNOWLEDGMENTS**

The authors thank Susan DeHaven and Elizabeth Jenkins for their assistance in the collection of these data. We also thank Rosemary Hennesy and Margie McMoore for their help in tabulating and analyzing these data.

**APPENDIX 11**  
**Brain Weight and Morphometric Historical Control**  
**Study for Day 10 and Day 12 Pups**

Morphometric Measurement Validation Study  
Comparing Day 10 and Day 12 Pups



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**NEUROPATHOLOGY REPORT**

**MORPHOMETRIC MEASUREMENT VALIDATION STUDY COMPARING  
DAY 10 AND DAY 12 PUPS**

**TESTING FACILITY**

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**CONSULTING PATHOLOGY LABORATORY**

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(7 Pages)

**MORPHOMETRIC MEASUREMENT VALIDATION STUDY COMPARING  
DAY 10 AND DAY 12 PUPS**

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ARGUS  
PAGE 829Neuropathology Report  
Morphometry Validation Study  
Page 3 of 7**MORPHOMETRIC MEASUREMENT VALIDATION STUDY COMPARING  
DAY 10 AND DAY 12 PUPS****SUMMARY**

Gross and microscopic morphometric measurements were performed on the brains of a total of 20 rat pups. The brains were from five male and five female rats that were 10 days of age and from five male and five female rats that were 12 days of age. The ages of 10 and 12 days were chosen to demonstrate that the measurement data from the selected neuroanatomic areas would reveal age-related developmental difference and might, therefore, be predictive of either an *in utero* neurotoxic effect or a delay in brain development. Two gross and seven microscopic measurements were taken.

The mean values for all of the neuroanatomic measurements were higher for the 12 day-old rat pups than for the 10 day-old pups, but there was considerable overlap between the Day 10 and Day 12 rat pup data, with the greatest measurement for at least one 10 day-old pup being greater than the lowest measurement for one of the 12 day-old pups. However, in spite of moderate data overlap between the two age groups and the presence of moderate intra-age group variability in the measurements for each neuroanatomic location, statistically significant increases (Day 12 *versus* Day 10 rat pups) were found for the following brain regions: anterior-posterior length of the cerebrum, anterior-posterior length of the cerebellum, thickness of the frontal cortex, width of the caudate-putamen, and height of the cerebellar cortex.

**INTRODUCTION**

The purpose of this study was to validate an approach to the performance of the morphometric component of those developmental neurotoxicity studies in which no microscopic neuropathologic lesions were encountered. A total of nine gross and microscopic morphometric measurements were taken. Rather than subjecting pregnant rats to a variety of neurotoxic agents, the decision was made to initially perform the morphometric measurements on rat pups of two different ages. The evaluated brains were, therefore, from male and female rat pups that were 10 and 12 days of age. Rat pups of these two ages were selected, because most developmental neurotoxicity studies include evaluations of brains from rat pups that are 12 days of age. Furthermore, our desire was to validate the hypothesis that differences in the measured neuroanatomic areas would be predictive of delayed development of the brain. The evaluated animals were all Sprague Dawley (CrI:CD®BR VAF/Plus®) rats.

**MATERIALS AND METHODS****Study Overview and Group Assignments:**

The brains from a total of 20 Sprague Dawley (CrI:CD®BR VAF/Plus®) rat pups were evaluated. These brains were from five male and five female rats that were 10 days of age and from five male and five female rats that were 12 days of age.

**Dissection and Trimming Procedures:**

The brains had been immersion-fixed in 10% neutral buffered formalin (NBF) at the Testing Facility (Argus Research Laboratories, Inc.) and were received in NBF. These brains were intact and still within their cranial vaults. The calvaria covering the dorsum of each brain had already been removed to enhance fixation. The brains were removed and weighed. At the time of weighing, two linear measurements were taken from each brain using a Vernier caliper. These were: 1) a linear measurement of the cerebrum from the anterior to



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posterior pole, exclusive of the olfactory bulbs and 2) a linear measurement of the cerebellum extending from the anterior edge of the cortex to the posterior pole. Both of these measurements are in millimeters.

After the brains had been weighed and the gross measurements taken, each brain was divided in a standardized fashion into six coronal slices as detailed, below. The olfactory bulbs were also processed, resulting in a total of eight sections for each brain. The coronal slices and olfactory bulbs were placed into two cassettes, with the slices from the anterior portion of the brain being placed into Cassette #1 and the sections from the posterior portion of the brain being placed into Cassette #2.

The multiple coronal brain slices were prepared as follows:

- 1) The first cut was made half-way between the ventral base of the olfactory bulbs and the optic chiasm.
- 2) The second cut was made through the optic chiasm.
- 3) The third cut was made through the mammillary body just posterior to the infundibulum.
- 4) The fourth cut was made through the midbrain.
- 5) The fifth cut was made through the cerebellum just anterior to its mid point.
- 6) The sixth cut was through the anterior portion of the medulla.

The first four cuts were made starting from the ventral aspect of the brain. The fifth and sixth cuts were made from the dorsal surface. All of the coronal slices were placed anterior face down into the tissue cassettes (so that these anterior faces were sectioned).

Histotechnical Procedures:

The brain slices were processed according to standard operating procedures for paraffin embedding. The tissue blocks were sectioned on a rotary microtome set at a thickness of five micrometers and the resulting sections stained with hematoxylin and eosin.

Morphometry Procedures:

Nine linear morphometric measurements were taken on each brain. As described, above, two of these measurements were taken from the intact brains prior to trimming and processing. These gross measurements were performed so that there would be two dimensions with an anterior to posterior orientation. The other seven measurements were performed on the coronal histologic sections. The following microscopic morphometric measurements were performed using a calibrated ocular micrometer:

- 1) Thickness of the frontal cortex. This measurement was of the dorsal portion of the cerebral cortex within the coronal section passing through the optic chiasm and was taken at a magnification of 40x.
- 2) Thickness of the parietal cortex. This measurement was of the dorsolateral portion of the cerebral cortex within the coronal section taken through the optic chiasm. This measurement was also taken at a magnification of 40x.
- 3) Diagonal width (maximum cross sectional width) of the caudate putamen and underlying globus pallidus. This measurement was taken at a magnification of 20x and was performed on the coronal section passing through the optic chiasm.
- 4) Thickness of the corpus callosum at its mid point within the section passing through the optic chiasm. This measurement was taken at a magnification of 100x.
- 5) Thickness of the hippocampal gyrus. This measurement was performed on the dorsal to lateral portion of the dentate gyrus within the section taken just posterior to the mammillary body. Measurements were taken from the hippocampus from both sides of this brain slice and the median value recorded. This measurement was taken at a magnification of 40x.

6) Height of the cerebellum at the level of the deep cerebellar nuclei, including lobes 1 - 6 and extending from the roof of the fourth ventricle to the dorsal surface. (This is the maximum height of the cerebellum.) This measurement was taken at a magnification of 20x.

7) Thickness of the external germinal layer of the cerebellum. Because the thickness of this layer varied considerably from region to region, multiple areas were measured over the dorsum of the cerebellum and the median value recorded as one measurement. This measurement was taken at a magnification of 400x.

The gross anterior to posterior micrometric measurements of the cerebrum and cerebellar cortex are expressed in millimeters. The microscopic micrometric measurements were multiplied by appropriate factors to yield dimensions in micrometers. Both the brain weight and morphometry data were subjected to analysis of variance (ANOVA) testing.

### RESULTS AND DISCUSSION


Two of the nine measurements were taken from the intact brain prior to processing and sectioning. These measurements were taken for two reasons - 1) so that at least two anterior to posterior dimensions would be available prior to slicing of the brains and 2) to minimize any impact of histologic artifact such as tissue shrinkage caused by dehydration (during tissue processing) or separation of the lobules of the cerebellum, such as frequently occurs when the sections are floated on the warm water bath after sectioning. A variety of neuroanatomic areas were selected for measurement, including the cortices of the cerebrum and cerebellum, subcortical structures such as the caudate-putamen and hippocampus, and a major white matter tract (corpus callosum).

The brain weight data and the morphometric measurements for both the 10 and 12 day-old rat pups are presented in Table 1. Although both male and female rat pups were included in this study, the sexes of these pups had inadvertently not been recorded at the time that the brains were taken. Therefore, the data for the two sexes are combined in this table. As would be expected, the mean values for all of the measurements were higher for the 12 day-old rat pups than for the 10 day-old pups, but this mean value increase was rather minimal for some areas such as the corpus callosum and the external germinal layer of the cerebellum. Furthermore, for all nine areas measured, there was overlap between the Day 10 and Day 12 rat pup data, with the greatest measurement for at least one 10 day-old pup being greater than the lowest measurement for one of the 12 day-old pups. Some of this overlap may have to do with the fact that the data for the two sexes are combined. The hippocampal gyrus thickness was the most variable measurement. In fact, it was noted at the time of evaluation that there was considerable variation in this dimension even within the same brain section (*i.e.* from one side of the brain to the other).

In spite of moderate data overlap between the two age groups and the presence of moderate intra-age group variability in the measurements for each neuroanatomic location, statistically significant increases (Day 12 *versus* Day 10 rat pups) were found for the following brain regions: anterior-posterior length of the cerebrum, anterior-posterior length of the cerebellum, thickness of the frontal cortex, width of the caudate-putamen, and height of the cerebellar cortex.

## **CONCLUSION**

This limited study suggests that both gross and microscopic morphometric measurements should be taken in order to obtain both anterior to posterior and cross sectional dimensions from a variety of neuroanatomic regions. Increases in the thickness of the frontal cortex, the cross sectional width of the caudate-putamen (and underlying globus pallidus), and the height of the cerebellar cortex correlated best with brain maturation between Day 10 and Day 12. The hippocampal measurement was quite variable in this pilot study. However, measurements of the dentate gyrus may prove to be of value in situations where a neurotoxic agent may affect this region. It is important to bear in mind that the approach described in this validation report merely represents a screening technique for situations in which neuropathologic lesions are not present. Modification of this approach may be indicated whenever neuropathologic changes affect specific neuroanatomic regions.

  
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TABLE 1  
MORPHOMETRIC MEASUREMENT VALIDATION STUDY COMPARING  
DAY 10 AND DAY 12 PUPS

Day 10	Brain Weight	Ant/Post Cerebrum	Ant/Post Cerebellum	Frontal Cortex	Parietal Cortex	Caudate Putamen	Corpus Callosum	Hippocampal Gyrus	Cerebellum	Ext. Germinal Layer
1	1.237	12.6	3.9	1440	1368	1584	288	1008	3120	48
2	0.956	11.2	2.9	1248	1080	1680	192	744	2544	36
3	1.258	12.4	3.7	1416	1440	1902	336	768	2928	53
4	0.914	10.5	2.8	1176	1152	1920	288	888	2016	43
5	1.331	12.6	3.1	1200	1368	1920	197	840	3168	46
6	1.288	12.7	3	1272	1104	2208	259	768	3168	46
7	1.223	12.5	2.7	1344	1488	2304	288	1152	3072	46
8	1.225	13	2.9	1368	1056	1920	211	936	3216	38
9	0.879	10.8	2	1080	1104	1248	288	797	2544	43
10	1.28	13.4	2.8	1392	1440	2304	288	960	3360	43
mean	1.159	12.170	2.980	1293.600	1260.000	1899.000	263.500	886.100	2913.600	44.200
SD	0.172	0.979	0.527	117.820	174.631	332.641	47.806	129.816	417.260	4.849
Day 12										
1	1.296	12.6	2.7	1392	1320	2160	307	768	3552	36
2	1.455	13.3	3.2	1536	1488	2400	230	720	3888	38
3	1.552	13.7	3.5	1560	1512	2544	269	1056	4932	43
4	1.449	13.6	3.6	1560	1344	2400	240	960	3600	43
5	1.13	12.4	2.5	1392	1248	2016	317	768	2688	38
6	1.511	13.6	4.1	1440	1488	2448	326	1056	3888	41
7	1.399	13.7	3.5	1440	1464	2160	269	888	3792	53
8	1.469	13.4	4.2	1392	1440	2304	211	1008	3936	53
9	1.093	12.1	2.9	1320	1296	2112	211	768	3024	53
10	1.083	12	2.6	1296	1248	2256	269	912	2928	48
mean	1.3437	13.04	3.28	1432.8	1384.8	2280	264.9	890.4	3622.8	44.6
SD	0.1802375	0.688315	0.6033241	94.01277	104.3379	168.1904	42.05671	128.224803	641.35981	6.686636756
	*	*		**		**			**	

\* P less than or equal to 0.05

\*\* P less than or equal to 0.01